

# ON ASSIMILATORY INHIBITION

IN

## CHLOROPHYLLOUS PLANTS.

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PRESENTED TO THE

UNIVERSITY OF LEIPZIG

FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY.

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BY

ALFRED JAMES EWART.

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(5.)

LONDON:

1896.

[*Reprinted from the* JOURNAL OF THE LINNEAN SOCIETY.]

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On Assimilatory Inhibition in Plants. By ALFRED JAMES EWART, B.Sc. (Lond.); 1851 Exhibition Scholar; late Demonstrator of Botany in University College, Liverpool. (Communicated by R. J. HARVEY GIBSON, M.A., F.L.S.)

[Read 21st November, 1895.]

ASSIMILATION, like any other plant function, is liable to be modified by the external conditions under which a plant may happen to be. Thus as regards temperature, there is a minimal, optimal, and maximal point for assimilation, just as there is for respiration, though the optimal, minimal, and maximal points for the two functions do not necessarily coincide. Assimilation may, however, also be modified by internal conditions, about which very little is known. Thus Dehnecke\* came to the conclusion, mainly from theoretical deductions, that green, non-assimilating chlorophyll bodies having the function of a leucoplastid, and not of a true chlorophyll body, are of common occurrence in green plants. Similarly Jumelle† found that as the result of subjecting lichens and mosses to dry heat, a condition might be induced in them during which they had lost the power of assimilation, but still continued to respire. Further research in this direction being evidently necessary, I undertook, at the suggestion of Prof. Pfeffer, a series of investigations to determine the various causes by which this condition of what we may term assimilatory inhibition may be produced and the phenomena connected with it.

It was at once evident that the method used by Jumelle was not so well adapted for a research of this kind as is a microscopical method, allowing more rapid observations and more detailed examination of the cells or tissues affected to be made. There are three well established methods by which a microscopical determination of the evolution of Oxygen by green assimilating cells in the presence of light can be made. The first and most important of these is Engelmann's‡ well-known Bacterium

\* Dehnecke, "Ueber nicht assimilirende Chlorophyllkörper." Inaugural-Dissertation, Universität in Bonn (Cöln, 1880).

† Jumelle, "Recherches Physiologiques sur les Lichens," in *Revue Générale de Botanique*, vol. iv. 1892, pp. 114, 159, 220, & 259.

‡ T. W. Engelmann, "Neue Methode zur Untersuchung der Sauerstoffausscheidung pflanzlicher und thierischer Organismen," in *Bot. Zeit.* Bd. xxxix. (1881) No. 28.



method. The second is the reduced indigo test, first brought into prominence by Regnault and subsequently by Beyerinck \*. This method is, however, unsatisfactory, because it requires the presence of a slight excess of sulphuretted hydrogen or  $\text{NaHSO}_2$  in the reduced indigo solution; and when once the latter has become blue, it can only be reduced again by treatment with fresh  $\text{SH}_2$  or  $\text{NaHSO}_2$ . The poisonous action of the sulphuretted hydrogen, and the fact that once the colour returns the experiment ceases, continuous observations being possible only by adding fresh reduced indigo solution, form serious objections to the method. The last objection also applies to the third method, which has indeed found but little practical application, of using the conversion of reduced hæmoglobin into oxyhæmoglobin as a test for the evolution of oxygen.

The advantages of the Bacterium method are that it is much more delicate and easier to observe, the injurious influence of the Bacteria is almost *nil*, continuous observations are possible with the same preparations, and, lastly, the rapidity of the movement and number of moving Bacteria enable a relative estimation to be made of the amount of oxygen evolved, and hence of the intensity of assimilation. Throughout the investigation the Bacterium method was used as a test for the evolution or non-evolution of oxygen. The Bacterium employed was Cohn's *Bacterium Termo* (= *Proteus vulgaris*, Hauser; = *Bacillus liquefaciens vulgaris*, Beyerinck), slope or stab cultures being made on Bouillon-Agar, and allowed to develop at a temperature of  $25^\circ\text{C}$ . In actual use the cultures should never be more than 1 to 2 weeks old, and the Bacteria should be taken from the edges of the growth, where it is of more recent formation, and hence consists almost entirely of motile forms.

Great care must always be taken to ensure the absolute purity of the cultures and their entire freedom from anærobic forms. This is readily ascertained by mounting the Bacteria in a drop of sterilized nutrient solution, and, after making certain of the absence of all air-bubbles, ringing the cover-slip with either pure vaseline, or vaseline stiffened with a little wax or paraffin, according to the temperature of the room.

If the culture is a pure one, the movement of the Bacteria ceases as soon as the enclosed dissolved oxygen is exhausted, and if any Bacteria continue to move after having been for one hour

\* Beyerinck, in Bot. Zeit. 1890, p. 742.



in a properly ringed and closed cell, then the culture is impure, and anærobic forms, which can move in the absence of oxygen if food material be present, or partial ærobes, such as certain *Spirilla*, which can move in the presence of an infinitesimal trace of oxygen, are present. The *Bacterium Termo* form used, in ordinary tap-water, containing a slight percentage of salts and but a mere trace of nutriment, continues to move as long as oxygen is present, and may remain living and motile in the absence of oxygen for 1 or 2 days, moving as soon as oxygen is admitted or generated. The sensitivity of the Bacteria, however, undergoes, with regard to oxygen, in 12 hours a distinct, and in 24 hours a marked alteration, they becoming less sensitive to the absence and more sensitive to the presence of minute traces of oxygen, *i. e.* partial starvation increases their sensitivity to oxygen. Finally, in the absence of all nutriment, *B. Termo* ceases to move in the presence of oxygen and becomes quite immotile. The maximal and minimal temperatures for movement are widely separated, the optimum temperature lying between 15° C. and 30° C. Above and below these limits the movement is not quite so active, but continues quite actively in the presence of oxygen on the one hand as far as zero, and on the other up to 40° C.

When a cell or tissue is examined by the Bacterium method to detect the presence or absence of the power of assimilating and evolving oxygen, it is naturally important to determine, especially if comparative experiments are to be made, to what extent the evolution of oxygen from the free surface of the cell is modified by the nature of the enclosing cell-membranes or by the surrounding fluid. Is, for example, oxygen evolved more rapidly through uncuticularized than through cuticularized epidermis? To settle this point, isolated hairs of *Gynura aurantiaca* were examined in a closed cell with Bacteria exposed to light. The hair-cells contain a purple dye dissolved in the cell-sap, which fades as the hair grows older, and in the lining plasma rather small and not abundant chlorophyll grains are present. A distinct though not strong evolution of oxygen is shown by all normal chlorophyll-containing hairs, whether the sap is purple or colourless. If the hair has separated exactly at the partition-wall between two cells, it can be seen that the evolution of oxygen takes place with equal activity from all surfaces of the cell. If the torn remains of a basal cell remain attached to the lower end of the hair, the



Bacteria move much more actively inside it, to and fro from the partition wall, than they do elsewhere. This is, however, because a certain amount of nutritious substance is present in such cases, and the Bacteria move more actively in the presence of dissolved nutritious substance and oxygen than in the presence of oxygen in water only. In many cases, on exposure to strong light the chlorophyll grains accumulate for the most part on the partition walls of the hair-cells, and in such separated end cells the evolution of oxygen is naturally more marked from the end wall than from the side walls. Epidermal hairs of *Cucurbita Pepo* and epidermal cells from *Allium* and *Aspidium* gave similar results, the chlorophyll grains being rather more numerous and the evolution of oxygen correspondingly more active. In the young leaves of *Cucurbita*, where the hairs are very abundant and large, the total amount of material assimilated by them must be fairly great, and is most active at a time when the assimilatory powers of the leaf are weak and undeveloped, but when it nevertheless demands stores of plastic material in greatest abundance.

It must be remembered that the conditions for the evolution of oxygen gas from a cell in water are not quite the same as they are in air. In the first case, the gas simply diffuses from a liquid in which it is abundant, through a damp membrane, to a fluid in which it is less abundant; in the second case, it is evolved from the surface of the cell because its partial pressure in solution is greater than in the mixture of gases, *i. e.* the atmosphere, by which the cell is surrounded. In ordinary aerified water, owing to the fact that  $\text{CO}_2$  is much more soluble in water than oxygen is, the evolution of the former gas will take place much more rapidly and readily as compared with the rates in air, from a cell in water, than that of the latter gas will. In the closed cell with Bacteria, on the other hand, since the partial pressure in solution of the dissolved  $\text{CO}_2$  is very high, whilst that of oxygen is very low, the latter gas will be evolved more rapidly and readily than the former. The difference in the rate at which gas is evolved persists only a very short time after transference from the one medium to the other, and ceases as soon as the amounts of gas dissolved in the cell-sap and permeating the cell are adjusted to their new levels, when the rate at which gas is evolved becomes, provided that the rate of production remains unaltered, the same as it was previously. A living cell or tissue kept in a closed cell-chamber with Bacteria will reach a condition of



equilibrium only when it contains less oxygen and more  $\text{CO}_2$  than it normally does, and hence arises the readiness with which prolonged experimentation under these conditions may produce asphyxiation in a non-assimilating tissue.

#### HEAT AND ASSIMILATION.

In investigating the effects of heat upon any plant function it is at once necessary to distinguish between dry heat and moist heat, the effects of dry heat being only possible to investigate on plants which can withstand desiccation.

##### *Effects of Desiccation and Dry Heat.*

The fact that the majority of seeds and spores can withstand even prolonged drying over sulphuric acid without being markedly affected is well known; and Schröder\* has placed on record an exhaustive series of observations upon the resistance which many vegetative plants show to similar desiccation. This resistance is shown by certain Hepatics (*Corsinia*, *Lunularia*, *Marchantia*); but in a more marked degree by certain Mosses (*Barbula muralis*, *Bryum cæspititium*, &c.), Lichens (*Sticta*, *Cladonia*, and *Evernia*), and Protophyceæ (*Nostoc*, *Scenedesmus*, *Hæmatococcus*, &c.). Jumelle's experiments also show that many mosses, lichens, &c. must possess considerable powers of resistance to dry heat and desiccation.

After collecting a number of plants of sufficient resistant power to desiccation, a series of preliminary heating experiments with mosses, of which the subjoined table is illustrative, were made. The plants were first air-dried, next dried in a desiccator for a day or two, and then placed in an oven for six hours and kept at a given constant temperature. The plants are then revived in a damp chamber, and leaf preparations from all parts made with Bacteria to test for assimilation, as well as being examined by plasmolysis before or after the presence or absence of assimilation has been ascertained.

The table very clearly shows that six hours' heating is too short an exposure to produce a marked inhibitory after-effect upon the function of assimilation. It is only when the temperature is extremely high that a complete and prolonged inhibition of assimilation is produced, and in such cases the greater number

\* Schröder, Bot. Untersuch. Tübingen (Pfeffer), Bd. ii. Heft 1 (1886).



TABLE A.

| Des. dried<br>plants for<br>6 h. at:— | 50° C.                                                                 | 60° C.                                                                                                                            | 70° C.                                                                                                                                                                                                      | 80° C.                                                                                                                                                                                                   | 90° C.                                                                                                                                                           |
|---------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Grimmia conferta</i> .             | All living, and assim. normal.                                         | All leaves living, but assim. weaker than normal. After 1 day in D. Ch. normal assim. returns.                                    | Younger leaves living and assim.; older living leaves assim. very weak. Plants remain living and recover.                                                                                                   | Apical leaves living, and in parts show evol <sup>n</sup> of O. Normal assim. in all living leaves in 1 day.                                                                                             | Yg. leaves and leaf-cells in parts still living, but no assim. & no recovery. A few plants form new sprouts on planting. At 95° C. all plants are killed.        |
| <i>Ceratodon purpureum</i> .          | Leaves all living, but evol <sup>n</sup> of O at first weak or absent. | All leaves living but no assim. After 4 h. in D. Ch. weak evol <sup>n</sup> of O, in 1 day assim. is of normal activity.          | Plants living, but no assim.; normal after 1 day in D. Ch.                                                                                                                                                  | Younger leaves living. No assim. In from 1 to 2 days in D. Ch., normal assim. in all living leaves.                                                                                                      | Plants all dead, and no recovery.                                                                                                                                |
| <i>Bryum caespitium</i> .             | Living, and assim. normal.                                             | Most leaves and leaf-cells living. Rather weak evol <sup>n</sup> of O, normal after 1 day in D. Ch.                               | Apical tuft living and showing fairly active assim. In older leaves more or less cells living and plasmolysable, but no assim. After 1 day in D. Ch. all living cells show distinct evol <sup>n</sup> of O. | Younger leaves all or most cells living. No assim., but in 1-2 days in D. Ch. normal assim. in all living cells. Most plants recover and resume growth.                                                  | Plants all dead, and no recovery.                                                                                                                                |
| <i>Barbula muralis</i> .              | Living, and assim. normal.                                             | Leaves for most parts living, assim. mostly weak, but in 1 day normal.                                                            | Younger leaves living, and leaf-bases show fairly active assim.; but outer parts none. In 1 day active assim. in all living leaves.                                                                         | Few leaves and leaf-bases living, and in some of latter a very faint evol <sup>n</sup> of O is shown. After 2 days in D. Ch. no assim., though plenty of cells are still living. Plants all finally die. | Plants all dead, and no recovery.                                                                                                                                |
| <i>Orthotrichum affine</i> .          | Living, and assim. normal.                                             | Leaves all living, but assim. weaker than normal. After 1 day in D. Ch. normal assim. returns.                                    | Younger leaves living and assim.; older living leaves, assim. weak. Remain living and resume growth on planting.                                                                                            | Younger leaves living; assim. in parts fairly active, in parts slight or none. In 1 day all living leaves show normal assim.                                                                             | Cells in parts of younger leaves living & plasmolysable, but no assim. and no recovery; in a few days all are dead. Heated to 95° C. plants are entirely killed. |
| <i>Dicranum scoparium</i> .           | Living, and assim. normal.                                             | Older leaves dead. Apical leaves living. Weak evol <sup>n</sup> of O, which in 1 day is normal. Plants recover and resume growth. | Young leaves of apical tuft living, in parts faint evol <sup>n</sup> of O. Plants do not recover, but on planting slowly die.                                                                               | All leaves dead.                                                                                                                                                                                         |                                                                                                                                                                  |

N B.—Evol<sup>n</sup> of O=evolution of Oxygen; assim.=assimilation; D. Ch.=Damp Chamber.



of the leaves are killed ; and even those which remain living and show plasmolysis on moistening may have their vitality so injuriously affected that they die without any return of assimilation taking place. One fact may at once be noticed, though it will appear again and again in the future experiments, and that is, that the stoppage of assimilation does not involve any perceptible change whatever in the assimilatory apparatus. Of two cells of precisely similar microscopical appearance, one may be assimilating and the other not.

The table is more of value as showing the extreme resistance which some Mosses show in the dried condition to short periods of heating at high temperatures, than as showing any marked production of assimilatory inhibition. Thus in *Grimmia* and *Orthotrichum* living cells may still be present after heating for 6 hours to 90° C., and may remain living for some days though they have lost all power of assimilation and recovery. In order to obtain the full resistant power of the plants, the air in which they are heated must be perfectly dry. This is well shown by the increased resistant power shown by *Grimmia conferta* under the conditions of the following experiment :—Desiccator dried plants are heated in a CaCl<sub>2</sub> desiccator for 6 hours at 95° C.; after moistening, a few of the younger leaves and leaf-cells are found to be living and plasmolysable, but show no assimilation and no recovery. On planting, however, a few plants form new sprouts, *i. e.* under these conditions the plant withstood a temperature 5° C. higher than in the Table A. That the presence of oil has little or nothing to do with the power of resistance to heat is shown by the fact that *Dicranum*, the protoplasm of which contains plenty of oil, is not more resistant, and is in many cases less resistant, than other Mosses in which such oil is absent.

It must be remembered that whenever Assimilation is said to be absent, what is really meant is that under optimal illumination in the presence of CO<sub>2</sub> no evolution of Oxygen can be detected. Assimilation and evolution of Oxygen are not necessarily synonymous, very weak assimilation may be masked by the concomitant respiration. Boussingault \* and Holle † state that green leaves in darkness evolve from  $\frac{1}{20}$  to  $\frac{1}{30}$  the amount of

\* Boussingault, 'Agronomie, Chimie Agricole et Physiologie,' vol. iv. 1868, p. 329.

† Holle, in 'Flora,' 1877, pp. 162 and 186.



CO<sub>2</sub> that they can assimilate in the same time under optimal illumination. Hence the possibility of error introduced by using the evolution or non-evolution of oxygen as a test for the presence or absence of assimilation is at ordinary temperatures but slight. Practically, whenever a cell examined by the Bacterium method under optimal illumination shows no evolution of oxygen, the power of assimilation is absent. At high temperatures, however, since respiration, according to Kreusler\*, and Bonnier and Mangin†, goes on continually and more and more rapidly, increasing with each rise of temperature, whilst according to Kreusler the assimilatory curve sinks, the possibility of a power of assimilation being present, though no evolution of oxygen can be detected, is much increased. In the following pages all the observations were, with a few exceptions on pp. 386, 387, made at a temperature not above 20° C. In any case, what is certain is that where no evolution of oxygen is taking place, no increase of plastic material can occur, though the waste of substance by respiration may be partially compensated for by weak assimilation. In actual experimentation it has been found that an assimilation and evolution of oxygen, which are imperceptible by the gas analytical method, can be readily detected by the more delicate and discriminatory Bacterium method.

When a preparation is made of a living leaf in which assimilation has been temporarily inhibited, in almost all cases the Bacteria come to rest sooner in the neighbourhood of the leaf than out in the open field. This is because the leaf, though it no longer assimilates, still respire, and the oxygen dissolved in the enclosed water is sooner exhausted in its immediate neighbourhood than it is further away. That such stoppage is not due to the extrusion of mucilage or other substance, exercising a retarding influence upon the movement of the Bacteria, by the leaf, is shown by the fact that the Bacteria move longer in the neighbourhood of a freshly-killed leaf than they do out in the open field.

A normal moss-leaf may remain living and assimilating, in water in a closed chamber with Bacteria, exposed to diffuse daylight for weeks. When, however, assimilation has been temporarily inhibited, the return is slower in a mounted leaf than in

\* Kreusler, "Beobachtungen über die Kohlensäure-Aufnahme und -Ausgabe der Pflanzen," in Landw. Jahrb. 1887, Bd. xvi. pp. 711-715.

† Bonnier and Mangin, in Ann. d. Sc. Nat. 7 sér. ii. 1885, p. 378.



one which remains in damp air attached to the parent plant, and in some cases may not take place at all. In water *Bacterium Termo* is to be regarded simply as an absorber of oxygen and producer of  $\text{CO}_2$ . That in water it exercises no other injurious poisonous influence upon the leaf-cells is shown by the long period of time during which a moss-leaf may remain living in a closed cell with Bacteria. Similarly the shock of removal from the parent plant is so slight as to be negligible. Hence the slower recovery of the separated leaf in a closed cell is due partly to the lack of oxygen, and perhaps partly to deficient nutrition.

The return of assimilation and recovery of the leaf need not necessarily be followed on the same leaf, but can be traced by making successive preparations at given intervals of time from neighbouring leaves. It is the age and position of the leaf which determine its resistant power; each leaf behaves similarly to its immediate neighbours, though between the basal and apical leaves wide differences in behaviour and resistant power may exist. As regards dry heat and desiccation it is the young leaves which are most, and the old leaves least, resistant. As the adult leaves grow older, their resistant power to dry heat, as well as their assimilating powers, becomes lessened.

To obtain good results, it is better to heat for a longer time at a lower temperature. The results of such heating are shown in the columns of Table B, from which several interesting conclusions can be deduced. A comparison between (a) and (b) and (c) and (d) shows that strong desiccation previous to heating lowers the plant's resistant powers, but that the drier the atmosphere in which the actual heating takes place the less is the plant affected. Columns c, d, and e show that the duration of the exposure is at least as important as the temperature to which the plant is heated, in producing a prolonged inhibitory after-effect upon assimilation. This after-effect is more readily produced in the older than in the younger leaves. Though it may take days before assimilation becomes quite normal, nevertheless in the young leaves assimilation is rarely completely absent for more than a few hours. Under the same exposure a more prolonged inhibitory after-effect is produced in *Dicranum* than in *Bryum* and *Orthotrichum*.

Table C gives the results obtained with various Chloro- and Cyanophyceæ. The plants are collected as pure as possible, dried in bulk, and then kept in thin flakes on watch-glasses in



the oven at the particular temperature required. They are then moistened, part at once examined, and the rest kept in the damp chamber, and portions examined from time to time. Since when a closed cell preparation with Bacteria is made, the Bacteria do not exhaust the enclosed dissolved oxygen and come to rest for a few minutes, it follows that however rapidly and carefully the preparations are made, sure and certain results are only given a quarter of an hour after the plants have been moistened and the preparations made. If examined before this, the Bacteria may be moving, not because any evolution of oxygen has taken place, but because the oxygen originally present has not yet been exhausted.

The plants in Table C were previously tested to make certain that they could withstand desiccation. After being dried in air at 20° C., and kept in a desiccator for a day or two, they were found all to remain living, and to show at once on moistening an active power of assimilation. Unicellular plants form very good working material, as they are hardly at all affected by manipulation, and each cell can be examined separately. The only precautions necessary are to avoid pressure and excess of either Algal material or Bacteria.

The results obtained are, as regards the inhibition of assimilation, similar to those obtained with Mosses, but the experiments are of especial interest as enabling a comparison to be made between Algal cells when free, and when combined with Fungi to form Lichens.

Table D gives the main results of the experiments with Lichens and with a vascular cryptogam, *Selaginella lepidophylla*. The resistance which the latter plant offers to drying is well known; and Bert and Bureau\* mention that it is able to withstand heating to 60° C., the duration of the exposure not being given.

A glance at the table shows that *Selaginella lepidophylla* is markedly affected by a day's heating at 55° C., and is killed after a day (24 hours) at 60° C. For the experiments living branches, the leaves of which showed an active power of assimilation, were cut off, allowed to roll up and become air-dried, and then heated to the required temperature. The first leaves to be affected by

\* Bert and Bureau, "Note sur la reviviscence du *Selaginella lepidophylla* et du *Ceterach officinarum*," in Comptes-rendus de la Société de Biologie, 1868.



TABLE B.

|                             | a. Air-dried, and open in oven for 2 days at 50° C.                                                                                                                                                                                                  | b. Desicc. dried and in Desiccator for 2 days at 50° C.                                                                                                                                                                                                    | c. Desicc. dried and in Desiccator for 5 days at 50° C.                                                                                                                                                                                                            | d. Desicc. dried, but open in oven for 5 days at 50° C.                                                                                                                                | e. Desicc. dried and in Desiccator for 2 days at 60° C.                                                                                                                                                                                                                                                                                                            |
|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Dicranum scoparium.</i>  | Exposed older leaves, no assim., and many cells dead. Younger leaves, all cells living and weak assim. Central leaves of apical tuft assim. almost normal. After 1 day in D. Ch., normal assim. in younger leaves; in 2-3 days, normal in all cases. | Older leaves with rather more dead leaf-cells than in (a). Weak assim. in central leaves of apical tuft; in rest of leaves none. In 1 day normal assim. in young leaves, but in older leaves still none. In 2-3 days assim. is shown by all living leaves. | In older leaves many cells are dead, but in young leaves all are living. No assim. In 5-6 h. slight evolution of O, which in 1 day is weak to moderately active. After 2 days many of the older leaves die without assim. returning; but in rest assim. is normal. | Very many leaves and leaf-cells killed. No assim. Many plants and all older leaves die, but in the rest the apical tufts remain living, assim. returns, and in 3 days is about normal. | Apical leaves living, and show weak assim. Older leaves plenty of cells living, but no assim. After 1 day in D. Ch. these are mostly still living, but no assim.; in young leaves active assim. After 3 days assim. still quite weak in many leaves. After 5 days all still living leaves show normal assim.                                                       |
| <i>Orthotrichum affine.</i> | Almost all leaf-cells living, but only slight assim., and this mainly in patches; neighbouring parts, all cells of which are living, showing no assim. After 1 day in D. Ch. normal assim. returns.                                                  | No perceptible difference from (a).                                                                                                                                                                                                                        | Most leaves and leaf-cells living. No assim. In 1-2 h. in D. Ch. weak assim. in some leaves, in 5-6 h. stronger, and in 2 days normal assim. in all living leaves.                                                                                                 | Most leaves and leaf-cells living. At first no assim., in 1-2 h. weak assim. in few leaves; fairly active in 1 day in most cases, and in 2 days normal.                                | Younger leaves living, assim. very weak. Older leaves, more or less leaf-cells are dead; no assim. In 1 day assim. fairly active in young leaves, weak or absent in rest. In 3 days normal assim. in nearly all living leaves. Many leaves with only patches of living cells finally die without assim. returning, though they may remain living for several days. |
| <i>Bryum caespitium.</i>    | Few leaf-cells in older leaves dead. Assim. weak or almost imperceptible. After 1 day in D. Ch. normal assim. in all cases.                                                                                                                          | Rather more leaf-cells dead. Assim. weak or imperceptible. Normal assim. after 1 day in D. Ch.                                                                                                                                                             | Many leaf-cells dead, especially in lower older leaves. No assim. In 3-4 h. faint assim. in some leaves, stronger after 1 day. After 2 days in D. Ch., all living leaves show normal assim.                                                                        |                                                                                                                                                                                        | Most leaves and leaf-cells living, but no perceptible assim. In 1 day weak assim., which in 3 days is fairly active. In many leaves no recovery of assim. takes place, though such may remain living for several days.                                                                                                                                             |



TABLE C.

|                                 | 1 day at 40° C.                                                                                                                  | 2 days at 40° C.                                                                                          | 1 day at 45° C.                                                                                                                                          | 1 day at 50° C.                                                                                                                                                                                                                                                                                                                                                                      |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Scenedesmus acutus</i> .     | In $\frac{1}{2}$ h. after moistening very weak evol <sup>n</sup> of O; in 1 h. stronger, but still weak; in 2-3 h. about normal. | After 5 h. in D. Ch. still no evol <sup>n</sup> of O; distinct but weak in 10 h.; normal in 1 day.        | Most cells dead, few living. After 1 day still no assim. In 3 days normal evol <sup>n</sup> of O. in all living cells. These were perhaps resting cells. | All cells dead. No recovery.                                                                                                                                                                                                                                                                                                                                                         |
| <i>Pleurococcus</i> , sp.?...   | As in <i>Scenedesmus</i> .                                                                                                       | Many cells killed; in rest no evol <sup>n</sup> of O perceptible till after 1 day in D. Ch.               | As in <i>Scenedesmus</i> .                                                                                                                               | All cells dead. No recovery.                                                                                                                                                                                                                                                                                                                                                         |
| <i>Glæocapsa</i> , sp.?.....    | Weak evol <sup>n</sup> of O in $\frac{1}{2}$ h.; in 1-2 h. normal assim. returns.                                                | In $\frac{1}{2}$ h. faint evol <sup>n</sup> of O, in 1 h. stronger, and in 2-3 h. about normal.           | Many cells killed, but many living. After 8 h. no evol <sup>n</sup> of O, but in 1 day evol <sup>n</sup> of O about normal.                              | All cells dead. No recovery.                                                                                                                                                                                                                                                                                                                                                         |
| <i>Rhaphidium polymorphum</i> . | As in <i>Scenedesmus</i> .                                                                                                       | Only few cells living and these do not show perceptible evol <sup>n</sup> of O till after 1 day in D. Ch. | All cells killed. No recovery.                                                                                                                           | The Algae on moistening, even if entirely living, do not take up so much water as formerly. This is simply due to a change in the gelatinous substances accompanying such Algae ( <i>Nostoc</i> , <i>Glæocapsa</i> ), and formed by them. The Algal cell and its integral coats swell up to their normal size, and absorb as much water as formerly, after an hour or two if living. |
| <i>Merispermædia</i> , sp.?     | As in <i>Glæocapsa</i> .                                                                                                         | In $\frac{1}{2}$ h. faint evol <sup>n</sup> of O, in 2 h. moderately active, and in 3-4 h. about normal.  | All cells killed. No recovery.                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                      |
| <i>Nostoc</i> , sp.? .....      | Very weak evol <sup>n</sup> of O in $\frac{1}{2}$ h.; in 1 h. still weak; in 2-3 h. about normal.                                | After $\frac{1}{2}$ h. still no assim., weak in 1 h., and in 3-4 h. about normal.                         | All cells killed. No recovery.                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                      |



TABLE D.

|                                  | 1 day at 45° C.                                                                                                                                                     | 2 days at 45° C.                                                                                                                                                                                                                                                                               | 3 days at 45° C.                                                                                                                                                                                                                                                                           | 1 day at 50° C.                                                                                                                                                                                                                                                    | 2 days at 50° C.                                                                                                             | 1 day at 55° C.                                                                                                                                                                                                                                                                       |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Selaginella lepidophylla.</i> | Leaves all living and Chl. grains normal. After 1 h. no evol <sup>n</sup> of O; in 2 h. weak, in 4 h. moderately active, and in 8 h. normal evol <sup>n</sup> of O. | After 2 h. no evol <sup>n</sup> of O. In 4 h. weak, and in 8 h. about normal assim.                                                                                                                                                                                                            | Most leaves living. After 4 h. no evol <sup>n</sup> of O, in 8 h. weak, and in 1 day fairly active.                                                                                                                                                                                        | Many leaves killed, most living. After 4 h. no evol <sup>n</sup> of O. After 8 h. in D. Ch. weak in few leaves; after 1 day in all living leaves, and active in same. In 3 days fairly active assim. in all living leaves.                                         | Very few leaves living, but show no assim. and no recovery, though the leaves may remain living in D. Ch. for from 1-2 days. | Plenty of living leaves. After 8 h. very weak evol <sup>n</sup> of O in few cases. After 1 day weak evol <sup>n</sup> of O from most living leaves, in parts active. After 3 days moderately active in all living leaves.<br><br>60° C. for 1 day. No All leaves killed. No recovery. |
| <i>Evernia Prunastri.</i>        | Gonidia mostly living; few parts show weak evol <sup>n</sup> of O, mostly none. In 4 h. moderately active assim.; in 8 h. quite active.                             | No evol <sup>n</sup> of O, after 4 h.; in 8 h. faint traces in younger branches; in 1 day weak, and in 3 days fairly active. Most gonidia are killed, and after 1 week greater part of Lichen is seen to be dead, but in parts the gonidia are living, multiplying, and actively assimilating. | Nearly all plants killed. No evol <sup>n</sup> of O after 8 h. In 1 day in few young branches, more or less gonidia living and weak evol <sup>n</sup> of O; in 3 days moderately active; and in 1 week these branches are quite regenerated, and show quite active evol <sup>n</sup> of O. | After 8 h. no evol <sup>n</sup> of O, weak in a few cases in 1 day. In 3 days greater part of Lichen is seen to be dead, but in few branches gonidia are living and show fairly active evol <sup>n</sup> of O. After 10 days these branches are living and normal. | Plants all killed. No recovery.                                                                                              | Plants all killed. No recovery.                                                                                                                                                                                                                                                       |



|                                                                                                                                                        |                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>Gonidia all living, but at first only in a few parts is a weak evol<sup>n</sup> of O shown, mostly none. In 4 h. assim. is active in all cases.</p> | <p>No. evol<sup>n</sup> of O; in 4 h. feeble evol<sup>n</sup> of O from many younger branches. In 8 h. feeble evol<sup>n</sup> of O from most parts; in a few young branches moderately active. In 1 day assim. is normal. Practically all gonidia remain living.</p> | <p>No evol<sup>n</sup> of O. After 8 h. weak in few cases, mostly none. In 1 day mostly weak evol<sup>n</sup> of O; in parts none, and in parts moderately active. In 3 days active evol<sup>n</sup> of O in most part, but in a few older branches evol<sup>n</sup> of O is still weak, and here more or less gonidia are dead.</p> | <p>After 2 h. no evol<sup>n</sup> of O; in 8 h. weak in few cases. In 1 day fairly active assim. in most cases; in few still weak. In 3 days assim. quite active, practically all gonidia remain living.</p> | <p>In 4 h. no evol<sup>n</sup> of O. In 8 h. faint traces, or in few cases weak evol<sup>n</sup> of O. In 1 day moderately active evol<sup>n</sup> of O for most part, in many cases still weak or absent. In 3 days active evol<sup>n</sup> of O in almost all parts.</p> | <p>In 8 h. faint traces of assim. in few younger branches. In 1 day weak evol<sup>n</sup> of O, and in some younger branches moderately active. In 3 days mostly moderately active evol<sup>n</sup> of O; in parts quite active. A good many gonidia have been killed, and in some parts all.</p> |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

*Cladonia rangiferina.*

|                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                        |                        |                        |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|------------------------|------------------------|
| <p>In 8 h. faint traces of assim. in few younger branches. In 1 day mostly weak evol<sup>n</sup> of O, moderately active in many younger branches. In 3 days mostly moderately active evol<sup>n</sup> of O, and in parts quite active. Many of older plants and branches, all gonidia killed, and no recovery. The gonidia are sooner affected, and are less resistant when thinly scattered than when in large clumps.</p> | <p>No evol<sup>n</sup> of O after 1 day. After 3 days in D. Oh., most plant, all gonidia, dead, and fungal part also shows signs of death. Few plants with more or less living clumps of gonidia; these remain greenish, rest of lichen turns quite white. Such parts show weak or moderately active evol<sup>n</sup> of O; the gonidia are proliferating and masking the dead cells. In a week the clumps are larger, and many show quite active evol<sup>n</sup> of O; but only few plants, or parts, are regenerated in this way*.</p> | <p>No evol<sup>n</sup> of O after 1 day. After 3 days in D. Oh., most plant, all gonidia, dead, and fungal part also shows signs of death. Few plants with more or less living clumps of gonidia; these remain greenish, rest of lichen turns quite white. Such parts show weak or moderately active evol<sup>n</sup> of O; the gonidia are proliferating and masking the dead cells. In a week the clumps are larger, and many show quite active evol<sup>n</sup> of O; but only few plants, or parts, are regenerated in this way*.</p> | <p>1 day at 60° C.</p> | <p>1 day at 65° C.</p> | <p>1 day at 70° C.</p> |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|------------------------|------------------------|

*Cladonia rangiferina.*

\* It is possible that here the gonidia which survived were in a resistant resisting stage, the vegetative gonidia being all killed, and an evolution of oxygen being only perceptible when the former had begun to proliferate and multiply.



For purposes of comparison the following table is appended, giving the results of heating the same plants in the moist condition, which at once shows how enormously the presence of water lowers the resistant power to heat of a plant which is capable of withstanding desiccation.

TABLE E.

|                                                                       | 1 day at 35° C.                                                                                          | 1 day at 40° C.                                                                                                              | 1 day at 45° C.                                                                      |
|-----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <i>Cladonia rangiferina</i> .....                                     | Most gonidia living. Weak evol <sup>n</sup> of O, which in 1 h. is moderately, and in 3 h. quite active. | Gonidia living in parts only. No evol <sup>n</sup> of O. In 1 h. at 20° C. weak, and in 3 h. moderately active.              | All gonidia killed.                                                                  |
| <i>Evernia Prunastri</i> .....<br><i>Parmelia parietina</i> ..... }   | As in <i>Cladonia</i> .                                                                                  | All gonidia killed.                                                                                                          |                                                                                      |
| <i>Selaginella helvetica</i> .....                                    | All leaves living, and show active evol <sup>n</sup> of O.                                               | Nearly all leaves living. No evol <sup>n</sup> of O; fairly active in $\frac{1}{4}$ h., quite in $\frac{1}{2}$ h., at 20° C. | Most leaves living. No evol <sup>n</sup> of O after 1 h., weak in 3 h., active in 5. |
| In <i>Selaginella</i> , after 1 day at 50° C., all leaves are killed. |                                                                                                          |                                                                                                                              |                                                                                      |



heating are the outer ventral leaves; the inner dorsal leaves, being shielded to a certain extent by the inrolling of the dried branches, resist longer. The leaves of *Selaginella* always contain a little air, hence the preparations must be kept in darkness when first made until the Bacteria cease to move, then exposed to light and examined.

The Lichens are examined by means of thin sections, in which the assimilating activity of the gonidial cells can be determined without the Fungal respiration being able to exercise any disturbing influence. Bearing the greater delicacy and discriminating powers of the Bacterium method in mind, it is not surprising to find that by its help an assimilation and consequent evolution of oxygen can be detected in cases and under conditions in which, according to Jumelle, no such evolution of oxygen should have been perceptible.

Thus Jumelle mentions that in plants of *Cladonia rangiferina* kept air-dried for three months and then moistened and examined, respiration was much weakened, and assimilation had almost entirely disappeared. Plants of *Cladonia rangiferina* and *Evernia Prunastri*, however, after being kept air-dried in wide open-mouthed bottles in darkness at 15° C. to 20° C., were for the most part living. On moistening, at first no perceptible or a mere trace of assimilation was shown, but after half an hour a moderately active, and after one hour a quite active evolution of oxygen is shown by the gonidia.

As Jumelle does not state under what conditions his air-drying experiments were conducted, it is impossible to be certain whether or not other factors beyond the air-drying came into play in his experiments. Thus the temperature at which the air-dried plants are kept is a very important factor, and air-dried plants exposed to sunlight are much more rapidly injuriously affected than similar plants kept in darkness.

In addition to this, the resistance which the plants offer to drying is largely dependent upon their vegetative vigour when collected.

The plants of *Cladonia* experimented with in Table D were quite young and healthy, but the plants of *Evernia* were not nearly so vigorous. A few additional experiments made with some normally vigorous plants of *Evernia*, which were collected after the table had been completed, showed that with these more healthy and more resistant specimens an increased temperature



of nearly 5° C. was needed to produce the same results as previously.

For purposes of comparison a few of Jumelle's results with dry heat are here given :—

At 40° C.—*Evernia Prunastri*. After 1 day still assimilated ;  
after 2 days gave off more CO<sub>2</sub> than it absorbed ;  
after 3 days respired only.

„ „ *Glæocapsa* and *Palmoglæa*. After 1 day assimilation  
is but little greater than respiration ; after  
2 days all power of assimilation is lost.

At 45° C.—*Dicranum scoparium* after 1 day gives off CO<sub>2</sub> and  
absorbs O in sunlight.

„ „ *Glæocapsa* and *Trentepohlia* after 1 day respire  
only.

At 50° C.—*Evernia Prunastri*. After 1 hour still assimilates ;  
after 3 hours respiration is stronger than assimilation ; after 14 hours respire only.

„ „ *Cladonia rangiferina*. After 5 hours assimilation  
is still stronger than respiration, after 14 hours  
gives off CO<sub>2</sub> in sunlight.

At 55° C.—After 1 hour Lichens give off CO<sub>2</sub> in sunlight ;  
but after 7 days *Cladonia rangiferina* still re-  
spires, though with but  $\frac{1}{5}$  to  $\frac{1}{10}$  its pristine  
vigour.

„ „ *Orthotrichum affine* after 7 days showed almost  
normal assimilation.

At 60° C.—*Evernia Prunastri* after 9 hours still respired.

On comparing these results with those given in the four preceding tables, it will at once be seen that the temperatures mentioned by Jumelle as necessary to produce the results given are, except with *Orthotrichum* and *Glæocapsa*, where the results obtained coincide fairly well, much too low. This discrepancy does not indicate any lack of accuracy in Jumelle's observations, but simply emphasizes the fact that the gas-analytical method is not so delicate and discriminatory as the Bacterium method is.

Thus in *Dicranum* a power of assimilation is still present long after it should, according to Jumelle, have ceased. In the leaves of the apical tuft of this plant, owing to the relative difficulty with which gases will diffuse to and fro from the inner and more protected leaves, and to the shading from the light of the inner



leaves by the outer, a power of active assimilation may be present in the inner leaves and be readily detected by the Bacterium method, though none can be detected by the gas-analytical method. It is impossible by the latter method to make any allowance for the fact that commonly the different parts of the same plant may be differently affected by the same exposure. Thus in the young leaves, after a given exposure, a power of assimilation may still be present, but in older leaves absent, whilst in the oldest leaves many or all of the cells may be killed. As seen above, such a plant by the gas-analytical method appears to have no perceptible power of assimilation.

In the case of Lichens the error is still more increased by the fact that here the assimilation has to act against the respiration of both Fungus and Alga, and that the assimilating cells are normally more or less shaded from light by the fungal hyphæ with which they are surrounded or covered. The substance of a Lichen is always rather opaque, and even where the gonidia are on the outside of the thallus, the inner gonidia are largely shielded from light which falls on the outside of the thallus. Jumelle\*, as the result of his experiments upon the assimilation of Lichens, concludes that there is no maximum or optimum intensity of illumination for assimilation in Lichens, but that sunlight is always better than diffuse daylight. The explanation of this, however, may be that even when exposed to direct sunlight the Algal cells in an intact Lichen are for the most part exposed to an intensity of illumination not greater than that of bright diffuse daylight. With crustaceous Lichens, Jumelle finds that it is only when exposed to direct sunlight that they give off more oxygen than  $\text{CO}_2$ , the result being probably partly due to the opacity of the Lichen, and by no means indicating that the gonidia only assimilate actively when exposed to intense light.

When examined in the form of thin sections, it is found that with increasing intensity of illumination beyond bright diffuse daylight no further increase in the rapidity of the evolution of oxygen takes place. The assimilation of the algal cells, whether free or combined, in a Lichen remains the same, provided the other external conditions are alike. The amount of oxygen given off by any Lichen when assimilating is dependent upon

\* Jumelle, "L'Assimilation chez les Lichens," in *Comptes Rendus*, Paris, t. cxii. 1890.



(1) the relative number and nature of the gonidia and the intensity of light to which *they* are exposed; (2) the proportionate amount and respiratory activity of the fungal component; (3) the respiratory activity of the algal cells themselves. In testing a Lichen for its assimilating powers by the Bacterium method, (2) is eliminated, and it is possible to be always certain that the algal cells are optimally illuminated.

It is not easy to understand why with equally vigorous plants the resistance of the gonidia of *Cladonia* to dry heat should be so much more pronounced than those of *Evernia*. The algal cells in both are extremely alike, being a similar Protococcus form. In *Cladonia* the gonidia are scattered or in clumps, interwoven with loose hyphæ on the outside of the thallus, the thick clear hyaline layer of closely packed hyphæ being internal. In *Evernia* the latter layer is external and the gonidia form a dense layer immediately beneath it and are also scattered through the central loose hyphal network. The gonidia in the latter case are therefore better protected from desiccation than they are in the former, but are nevertheless less resistant. The explanation may be that the more exposed position of the apparently similar gonidia of *Cladonia* has caused them to become by adaptive modification more resistant to desiccation, and this to such an extent as to more than counterbalance their greater exposure.

To determine the resistance of the Fungal component is not at all easy. It is extremely difficult to say on immediate examination whether the Fungal hyphæ are dead or living. To plasmolytic methods they do not answer, and microscopical examination commonly fails at first to detect any perceptible difference between dead and living hyphæ, even where these are loosely woven as in the medulla. After being kept for several days in a damp chamber, the hyphæ of a dead plant begin to show various disintegratory changes, saphrophytic fungi begin to appear, the gonidia bleach, and the plants become much less water-absorbent, even in a damp atmosphere being more or less shrivelled. It is, however, always possible that in such cases the Fungal component was originally living, its death following only as a result of the death of the Algal component.

An exposure which kills all the gonidia seems also sufficient to destroy the vitality of the Fungus, for such plants if fed with sugar



solution show no signs of life\* ; and even if sprinkled repeatedly with water containing living Protococci obtained from specimens of the same Lichen, no regeneration and reorganization of the Lichen takes place as might be expected to occur if the fungal hyphæ were living. On the other hand when, as in many cases, the gonidia remain living only over a part of the thallus, such parts remain living and normal in appearance however long they may be kept under observation. Also in such areas many of the gonidia may be killed, only a portion remaining living ; and when this is the case after a few days to a week or more in such regions a multiplication and regeneration of the gonidia take place, the dead algal cells becoming less and less numerous as the living ones multiply.

Hence whenever the gonidia remain living, the Fungus remains living also, the Algal component being apparently the first to be killed. The conclusion, therefore, is that the resistant power of the Fungal component to desiccation and dry heat is slightly higher than that of the Algal component, but that the difference is very slight, it being impossible by drying or heating to entirely kill the one without also killing the other.

It is worthy of notice in this connection that the combination of Alga with Fungus seems to increase the resistant power of the former (compare tables C & D). Whether this is (*a*) a temporary modification due to the symbiosis, or (*b*) a merely mechanical protective effect of the enclosing fungal hyphæ, or (*c*) a special permanent modification induced by natural selection, is difficult to say. That (*b*) exercises some influence is certain, and that (*a*) is of some importance is rendered probable by the fact that the symbiotic condition does in many cases induce a change in the structure of the Alga, such as the breaking-up of chains of cells and the thickening of the cell-wall, which latter would readily account for an increased resistant power. Whether such temporary modification due to the environment can be rendered permanent by natural selection is an open question. In the case of the Fungal component a permanent modification, selective or acquired, one indication of which is the increased thickness of the hyphal cell-walls, fitting it for a dry habitat, has certainly

\* A. Möller ('Ueber die Cultur flechtenbildender Ascomyceten ohne Algen,' Münster, 1887) has shown that it is possible to grow and develop in suitable media the isolated Fungal components of Lichens.



taken place, for the vegetative mycelia of ordinary fungi are readily killed by drying.

Certain of the conclusions which Jumelle draws from his experiments on the effects of dry heat on assimilation cannot be passed over without criticism. He concludes that (*a*) heating alters chlorophyll whether the cell contains water or not, but that the protoplasm remains uninjured for a much longer period, and here the presence or absence of water is an important factor; (*b*) the stoppage of assimilation is a permanent one and is due to a destruction of the chlorophyll, the protoplasm remaining living and capable of respiration; (*c*) it is owing to this ready decomposition and destruction of the chlorophyll that lower green plants, although often capable of drying, offer little more resistance to dry heat than higher plants do; (*d*) chlorophyll is destroyed by heat before protoplasm is; (*e*) non-chlorophyllous plants are those which offer the greatest resistance to heat, and hence he concludes that the Alga in a Lichen is killed long before the Fungus is, which does not appear to be the case.

The stoppage of assimilation produced by dry heat is not necessarily a permanent one and is never accompanied by any visible alteration in the colour, form, shape, or condition of the chlorophyll bodies so long as the cell remains living, though of course it is always possible that an invisible alteration may occur. The presence or absence of water is of just as much importance in the preservation of the vitality of the chlorophyll grain as in that of the protoplasm. The presence or absence of chlorophyll has little or nothing to do with the resistant power of plants to dry heat. Non-chlorophyllous plants are not necessarily more resistant to dry heat than chlorophyllaceous plants. It is doubtful whether any plants, chlorophyllous or non-chlorophyllous are, in the vegetative condition, more resistant to heat than the mosses in the experiments given in Table A. Chlorophyll is certainly not destroyed by heat before protoplasm is. In certain cases, it is true, a cell may remain living for a few hours or even days without the power of assimilation returning, but microscopical examination fails to detect any change in the assimilatory apparatus until the death of the cell takes place.

It is very possible that both the duration and degree of the desiccation to which the plants are submitted are of importance in inducing the inhibitory after-effect upon assimilation. The preceding tables show the importance of the first factor, and that of



the second appears to be shown by the following data:—The upper leafy portions of the stems of *Dicranum scoparium* are kept air-dried for 3 months at 18° C. to 20° C. The leaves and leaf-cells are for the most part living, and on making cell-preparations with Bacteria show as soon as examined an active evolution of oxygen, *i. e.*, the absorption of water is almost immediately followed by a resumption of assimilation even after so long a dormant period. A sample of such dried plants contained 8.18 per cent. of water (calculated by keeping a known weight in a  $\text{CaCl}_2$  desiccator at 100° C. to 105° C. till no further loss of weight takes place). After drying in desiccator for several days, until the weight shows no further appreciable diminution, the plants still contained 1.78 per cent. of water.

Another sample of fresh plants contained 53.7 per cent.; after being air-dried for a few days contained 15.88 per cent.; after being in a desiccator at 35° C. till the weight remained constant, contained 2.07 per cent. of water.

Plants of *Cladonia rangiferina* kept air-dried for 3 months at 15° C. to 20° C. are almost unaffected, the gonidia being almost all living. If moistened and rapidly examined, at first no perceptible or a mere trace of an evolution of oxygen is shown, but this after  $\frac{1}{2}$  hour is moderately active and in 1 hour is quite active. The plants contained 14.55 per cent. of water; after being kept for 1 day at 45° C. they contained 6.9 per cent., after 2 days 6.84 per cent., after 3 days 6.76 per cent., and after a 4th day at 50° C. 5.56 per cent.

On comparing these results with those in Table D, it can at once be seen that there is a distinct relation between the degree of desiccation and the inhibitory after-effect produced upon assimilation. The relation between the two is, however, by no means proportional, and whether it is causal or merely accidental it is impossible to say.

#### *Effects of Moist Heat.*

It is well known that at a certain maximal temperature a chlorophyllous cell or tissue ceases to assimilate. Thus with *Aspidium*, *Mimosa*, *Chara*, *Elodea*, and various Mosses at a temperature of from 37° C. to 39° C., though exposed to optimal illumination, no evolution of oxygen can be detected by the Bacterium method. It is of course always possible that assimilation may continue at temperatures considerably above those at which any evolution of



oxygen ceases to be perceptible, the latter being overpowered by the increased respiration before assimilation actually ceases. Thus Kreuzler\* found that assimilation begins to decrease at 30° C., at 45° C. is not entirely stopped, and at 50° C. is imperceptible (*Rubus* and *Ricinus*). The stoppage of assimilation is here a direct effect of the temperature to which the plants are exposed. To produce a stoppage of assimilation as an after-effect of the exposure, and persisting after the exposure has ceased, is by no means easy. If the exposure is severe the plant may be fatally injured and not recover at all; if less severe, it may be either unaffected or recover almost immediately. This is shown in Table F. The plants were exposed for 1 day at the temperature given, and after examination at the same temperature, brought to a temperature of 18° C. and kept under continuous observation.

TABLE F.

|                                                                                                                               | At 31° C. to 32° C.                                                                                                                                                                                  | At 36° C. to 37° C.                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Chara fragilis</i> .....                                                                                                   | Rotat <sup>n</sup> rapid. Evol <sup>n</sup> of O very weak, in $\frac{1}{4}$ h. at 18° C. mod. active, in $\frac{1}{2}$ h. quite active. Rotat <sup>n</sup> slightly slower.                         | Rotat <sup>n</sup> slightly slowed. No evol <sup>n</sup> of O. In $\frac{1}{4}$ h. at 18° C. weak, in $\frac{1}{2}$ h. mod., and in 1 h. quite active and rotat <sup>n</sup> normal. |
| <i>Elodea canadensis</i> .....                                                                                                | Very weak evol <sup>n</sup> of O. Few cells slow rotat <sup>n</sup> $\frac{1}{4}$ h. at 18° C., rotat <sup>n</sup> active, evol <sup>n</sup> of O mod. active, and in $\frac{1}{2}$ h. quite active. | No evol <sup>n</sup> of O or rotat <sup>n</sup> . In $\frac{1}{4}$ h. at 18° C. rotat <sup>n</sup> active, no evoln. of O, but in $\frac{1}{2}$ h. weak and in 1-2 hrs. active.      |
| <i>Selaginella helvetica</i> .<br><i>Oxalis Acetosella</i> .<br><i>Cystopteris fragilis</i> .<br><i>Metzgeria furcata</i> . } | Very weak evol <sup>n</sup> of O, in $\frac{1}{4}$ h. to $\frac{1}{2}$ h. at 18° C. mod. active, and in 1 h. quite active.                                                                           | No evol <sup>n</sup> of O, in $\frac{1}{4}$ h. weak, in $\frac{1}{2}$ h. mod. active, and in 1-2 hrs. quite active.                                                                  |
| <i>Orthotrichum affine</i> .<br><i>Parmelia parietina</i> .<br><i>Dicranum scoparium</i> . }                                  | Weak evol <sup>n</sup> of O, fairly active in $\frac{1}{4}$ h. at 18° C., quite in $\frac{1}{2}$ h.                                                                                                  | No evol <sup>n</sup> of O, weak in $\frac{1}{2}$ h., mod. active in 1 h., and quite in 2 hrs.                                                                                        |
| <i>Bryum caespititium</i> ...                                                                                                 | Rather weak evol <sup>n</sup> of O, quite active in $\frac{1}{4}$ h. at 18° C.                                                                                                                       | No evol <sup>n</sup> of O, weak in $\frac{1}{4}$ h. at 18° C., mod. active in $\frac{1}{2}$ h., quite in 1 h.                                                                        |

Table G shows that plants kept at 37° C. to 38° C. for 3 days, if kept moist and well supplied with oxygen, are all living, and almost no inhibitory after-effect upon assimilation has been produced.

\* Kreuzler, "Beobachtungen über die Kohlensäure-Aufnahme und -Ausgabe der Pflanzen," in Landw. Jahrb. 1887, Bd. xvi. pp. 711-715.



TABLE G.

|                               |                                                                                                                                              |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Selaginella helvetica.</i> | No evolution of O at 38° C., weak in $\frac{1}{4}$ h. at 20° C., fairly active in $\frac{1}{2}$ h., quite in 1 h.                            |
| <i>Oxalis Acetosella.</i>     | No evolution of O at 38° C., weak in $\frac{1}{2}$ h. at 20° C., moderately active in 1 h., quite active in 2 hrs.                           |
| <i>Chara fragilis.</i>        | Slow creeping rotation, which in 5 minutes is fairly active. No evolution of O at 38° C., weak in $\frac{1}{4}$ h. at 20° C., active in 1 h. |
| <i>Elodea canadensis.</i>     | No rotation, slow in 5 minutes and in 15 minutes fairly active. No evolution of O at 38° C., weak in $\frac{1}{4}$ h., quite active in 1 h.  |

If, however, plants are kept at a raised temperature in a limited supply of air for some time, a stoppage of assimilation, persisting for some time after the plant has been brought under normal conditions, may be fairly readily induced. The effect is really due to the plants being subjected to partial asphyxiation, and is more readily produced in Phanerogams than in Mosses, owing to the former plants being more sensitive to asphyxiation than the latter are. The results of heating under such conditions are shown in Table H, the young plants being kept in pots under closed bell-jars for a day at 37° C. to 38° C., examined at the

TABLE H.

|                                                                                                       |  |                                                                                                                                                                                                                                                                                                                                                    |
|-------------------------------------------------------------------------------------------------------|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Helianthus annuus.</i><br><i>Cucurbita Pepo.</i><br><i>Vicia Faba.</i><br><i>Ricinus communis.</i> |  | No evolution of O. After 1 h. at 20° C. still none. After 3 hrs. weak, in 6 hrs. fairly active, and in 1 day quite active.                                                                                                                                                                                                                         |
| <i>Zea Mays.</i><br><i>Aspidium violascens.</i>                                                       |  | No evolution of O, weak in 2 hrs., fairly active in 4, quite active in 1 day.                                                                                                                                                                                                                                                                      |
| <i>Phaseolus vulgaris</i> ...                                                                         |  | No evolution of O. Stems and undeveloped leaves only remain living. The former show after 1 day a distinct evolution of O.                                                                                                                                                                                                                         |
| <i>Lupinus albus</i> .....                                                                            |  | Cotyledonary and young undeveloped foliage leaves living. Protoplasm in many of cotyledonary cells aggregated in 3 to 6 spherical masses; when these fuse together, the cell is again plasmolysable. Chlorophyll grains normal, but frequently massed together. No evolution of O, none after 5 hrs., weak in 10 hrs., and in 1 day fairly active. |



same temperature, and then brought to 20° C. and kept under continuous observation.

In the first six plants all the leaves remain living and recover. In both *Lupinus* and *Phaseolus* the foliage leaves, which after a day or so at 20° C. are entirely dead, contained at first many plasmolysable cells with normal chlorophyll grains. These cells show no evolution of oxygen and do not recover, having permanently lost the power of assimilation.

A prolonged stoppage of assimilation produced as a result of moist heat alone can only be brought about when the temperature to which the plant is heated approaches dangerously near to its critical point (Table I). The plants were heated in darkness with a relatively abundant supply of moist air.

TABLE I.

|                               | 1 day at 40° C.                                                                                                                                                                                                | 1 day at 45° C.                                                                                                                              |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Cladonia rangiferina.</i>  | Gonidia mostly living. No evolution of O. In 1 h. at 20° C. weak evolution of O, which in 3 hours is moderately active.                                                                                        | Dead.                                                                                                                                        |
| <i>Oxalis Acetosella.</i>     | Most leaves living, few with brownish dead patches. Both no evolution of O, in 2 hrs. moderately active in former, in latter still weak after 5 hrs., moderately active in 1 day.                              | "                                                                                                                                            |
| <i>Dicranum scoparium.</i>    | Plenty of living leaves; chl. grains normal, but protoplasm somewhat attenuated. No evolution of O, after 1 day still imperceptible, in 2 days weak, and in 5 days active from all still living cells.         | "                                                                                                                                            |
| <i>Bryum caespititium.</i>    | Most leaves living. After 8 hours no evolution of O, in 1 day weak and in 2-3 days active in all living cells. As in <i>Dicranum</i> , many cells die without assimilation returning, but most plants recover. | "                                                                                                                                            |
| <i>Selaginella helvetica.</i> | No evolution of O, fairly active in $\frac{1}{4}$ to $\frac{1}{2}$ h. at 20° C., quite in $\frac{1}{2}$ to 1 h. Almost all leaves and leaf-cells living.                                                       | Most leaves living. No evolution of O, weak in 3 hrs., fairly active in parts in 5 hrs., in many still weak. In 1 day evolution of O normal. |



*Cold and Assimilation.*

The lowest temperature at which determinations can be made by the Bacterium method is just above zero at about  $1^{\circ}\text{C}$ . In all cases, unless otherwise mentioned, the frozen plants are thawed and examined at this temperature and then brought to a temperature of from  $15^{\circ}$  to  $20^{\circ}\text{C}$ . The following plants taken during January after 3 weeks continuous frost, the temperature often falling below  $-15^{\circ}\text{C}$ ., showed when examined no power of assimilation, though most of the leaves remained living:—*Ilex Aquifolium*, *Buxus sempervirens* var. *arborescens*, *Pinus montana*, *Taxus baccata*, *Thuja occidentalis*, *Juniperus Sabina*, *Cheiranthus Cheiri*, *Ulex europæus*, *Cytisus austriacus*. After 2 to 4 hours at  $15^{\circ}\text{C}$ . weak assimilation, in parts moderately active, is shown, and in from 8 hours to 1 day assimilation is quite active. A long latent period of recovery indicates that the exposure has very nearly reached the plant's limit of resistance.

Many herbaceous plants are able, if covered by snow, to retain last year's leaves alive and green during the entire winter. A series of such plants were examined in the middle of winter after having been covered by from 1 to 2 feet of snow for 4 weeks, when in all cases at first no assimilation was shown, weak to moderately active after 3–5 hours at  $15^{\circ}\text{C}$ ., and quite active in 1 day:—*Primula marginata*, *Helleborus lividus* and *H. guttatus*, *Poa pratensis*, *Catharinea undulata*, *Ceratodon purpureum*, *Hypnum triquetrum*, *Secale cereale*, *Triticum sativum*, *Opuntia Rafinesquii*. Certain herbaceous plants are able to withstand prolonged and severe exposure without being markedly affected. Thus the leaves of flowering plants of *Crocus*, after being kept frozen for a week at from  $-2^{\circ}\text{C}$ . to  $-5^{\circ}\text{C}$ ., showed on examination fairly active assimilation. After further exposure for 3 days to  $-5^{\circ}\text{C}$ . to  $-12^{\circ}\text{C}$ . they remained living, showed at first no assimilation, but after 1–2 hours at  $15^{\circ}\text{C}$ . weak, in 3 hours fairly, and in 5 hours quite active assimilation. Similarly, plants of *Sempervivum Braunii*, after being exposed for 3 weeks to a temperature varying from  $-5^{\circ}\text{C}$ . to  $-15^{\circ}\text{C}$ . were living but showed no assimilation, none after 3 hours at  $15^{\circ}\text{C}$ ., faint in 5 hours, and fairly active in 1 day.

The stoppage of assimilation caused by exposure to cold is not necessarily accompanied by any visible change in either chlorophyll or protoplasm, though changes may in certain cases be



produced in the colour of the chlorophyll grains by prolonged and severe exposure. This brown or yellow coloration commonly shown by Conifers, *Buxus*, &c. during winter has been most thoroughly investigated by Schimper, who finds that the brown coloration is due to a modification of the chlorophyll pigment caused by the combined effect of exposure to cold and light. In certain cases a yellow coloration is produced, the chlorophyll grains being quite pale or faintly yellowish. This, according to Haberlandt, is due to the light decomposing the chlorophyll and the low temperature preventing its re-formation. Schimper finds that the change of colour is due solely to a modification of the chlorophyll pigment, and is not accompanied by any destruction or disintegration of the chlorophyll bodies. Mechanical influences, pressure, section-cutting, or intense frost may cause the chlorophyll grains to disintegrate, but all such cells soon die and cease to be plasmolysable. One result of prolonged severe exposure to cold is to make the chlorophyll grains very liable to be disrupted and disintegrated. A chlorophyllous cell, which has been browned or turned yellow by the action of cold, can only become green again if the chlorophyll grains remain intact.

Browned specimens of *Buxus*, *Juniperus*, *Taxus*, *Abies*, and *Biota* became green again after being kept in a greenhouse at 10° C. to 15° C. for from 3 to 5 weeks.

None of the browned parts showed any perceptible power of assimilation even after being for several days in a greenhouse, but any parts with green and normal chlorophyll grains showed in from a few hours to a day a distinct power of assimilation. In deeply-browned specimens of *Juniperus communis* a weak evolution of oxygen can be detected as soon as the leaves begin to turn green, and a fairly active evolution of oxygen may be shown in leaves in which a slight trace of the brown colour is still perceptible. Pale yellow branches of *Thuja orientalis* begin to turn green in 3 weeks. After 4 weeks plenty of branches are still quite pale yellow and show no evolution of oxygen, but others though pale are distinctly green and show a weak power of assimilation. After 5 weeks fairly active assimilation is shown by leaves which are still slightly yellowish, and the chlorophyll grains, though green, paler than normal.

If the exposure inducing browning be too severe and prolonged the power of recovery is lost, but otherwise, provided the



chlorophyll grains remain uninjured and intact, the re-formation of the green pigment soon takes place, and this occurs more rapidly in entire plants than in experiments as above with isolated branches. As might be expected, the return of assimilation is coincident with the re-formation of the chlorophyll, and begins before the formation of the latter is complete. In many plants, however prolonged the exposure may be, no change of colour is produced in the chlorophyll so long as the cells remain living. Thus in *Ulex* and *Ilex*, at the end of winter all the exposed leaves or spines were brown, but the browning is here a post-mortem change and is a sign of death. It frequently happens that the different parts or even different layers of the same leaf may be differently affected. Thus in *Buxus*, at the end of winter many leaves have in the middle mesophyll layers normal green chlorophyll grains and show a distinct power of assimilation, those of the upper layer of palisade parenchyma being quite brown and showing no power of assimilation, whilst the cells of the lower spongy mesophyll, forming about one-third the thickness of the leaf, are all dead and shrunken. Such leaves in spring soon die and drop off. In most leaves the upper outer layers of the palisade parenchyma contain brown or greenish-brown chlorophyll grains, and show none or only very faint power of assimilation, whilst the less exposed tissue on the under-side of the leaf has distinctly green chlorophyll grains and shows a fairly active power of assimilation.

In order to compare the effect of prolonged exposure upon the subsequent powers of respiration of the plant with that upon assimilation, a few experiments, the results of which are given on pp. 392-393 were performed. The  $\text{CO}_2$  given off is absorbed by  $\text{KHO}$ , the decrease in the volume of air contained in the receiver giving approximately the amount of  $\text{CO}_2$  evolved. The readings are taken at the same temperature and pressure throughout, and the experiments are continued until the vitality of the specimens begins to diminish and the amount of  $\text{CO}_2$  evolved to decrease.



I. *Ulex europæus*.—Living green branches showed at first no perceptible evolution of Oxygen but in 3 hours at 15° C. weak and in 8 hours fairly active.

| 5 grammes green branches. |           |                                     |           | 5 grammes similar green branches. |           |                                     |             |
|---------------------------|-----------|-------------------------------------|-----------|-----------------------------------|-----------|-------------------------------------|-------------|
| Reading.                  | Time.     | CO <sub>2</sub> evolved per 30 min. |           | Reading.                          | Time.     | CO <sub>2</sub> evolved per 30 min. |             |
| 182 c.c.                  | 9.30 A.M. |                                     |           | 181 c.c.                          | 9.30 A.M. |                                     |             |
| 180.5 c.c.                | 12 „      | 0.3 c.c.                            | Light.    | 178.8 c.c.                        | 12 „      | 0.44 c.c.                           | } Darkness. |
| 178.5 c.c.                | 2.30 P.M. | 0.4 c.c.                            | Darkness. | 176.3 c.c.                        | 2.30 P.M. | 0.50 c.c.                           |             |
| 178.1 c.c.                | 5 „       | 0.08 c.c.                           | Light.    | 173.9 c.c.                        | 5 „       | 0.48 c.c.                           |             |
|                           | Next day. |                                     |           |                                   | Next day. |                                     |             |
| 157.7 c.c.                | 10 A.M.   | 1.2 c.c.                            | Darkness. | 161.5 c.c.                        | 10 A.M.   | 0.73 c.c.                           | } Light.    |
| 157.5 c.c.                | 10.30 „   | 0.2 c.c.                            | Light.    | 161.3 c.c.                        | 10.30 „   | 0.2 c.c.                            |             |
| 157.5 c.c.                | 3 P.M.    | 0.0 c.c.                            | Light.    | 161.3 c.c.                        | 3 P.M.    | 0.0 c.c.                            | Light.      |
| 156.5 c.c.                | 5 „       | 0.25 c.c.                           | Darkness. | 160.5 c.c.                        | 5 „       | 0.2 c.c.                            | Darkness.   |

II. *Thuja Standishii*.—Specimens taken after temperature had been at from -8° C. (day) to -15° C. (night) for several days, showed after 3 hours at 18° C. no power of assimilation, but after 5 hours weak and in 1 day fairly active assimilation in all cells with normal green or only slightly browned chlorophyll grains. All cells with quite brown chlorophyll grains, which are mostly in the outer layers of the leaf, show after 1 day still no assimilation.

| 8 grammes quite brown twigs. |           |                                     | 8 grammes green or very faintly browned twigs. |           |                                     |
|------------------------------|-----------|-------------------------------------|------------------------------------------------|-----------|-------------------------------------|
| Reading.                     | Time.     | CO <sub>2</sub> evolved per ½ hour. | Reading.                                       | Time.     | CO <sub>2</sub> evolved per ½ hour. |
| 181.5 c.c.                   | 3.30 P.M. | In darkness.                        | 180.0 c.c.                                     | 3.30 P.M. | In darkness.                        |
| 181.0 c.c.                   | 4 „       | 0.5 c.c.                            | 178.7 c.c.                                     | 4 „       | 1.3 c.c.                            |
| 180.7 c.c.                   | 4.30 „    | 0.3 c.c.                            | 178.1 c.c.                                     | 4.30 „    | 0.6 c.c.                            |
| 180.5 c.c.                   | 5 „       | 0.2 c.c.                            | 177.5 c.c.                                     | 5 „       | 0.6 c.c.                            |
| 180.3 c.c.                   | 5.30 „    | 0.2 c.c.                            | 177.0 c.c.                                     | 5.30 „    | 0.5 c.c.                            |
|                              | Next day. |                                     |                                                | Next day. |                                     |
| 169.5 c.c.                   | 10 A.M.   | 0.315 c.c.                          | 170.0 c.c.                                     | 10 A.M.   | 0.178 c.c.                          |
| 168.6 c.c.                   | 12 „      | 0.225 c.c.                          | 169.3 c.c.                                     | 11.30 „   | 0.23 c.c.                           |
|                              |           |                                     |                                                | 3rd day.  |                                     |
|                              |           |                                     | 160.4 c.c.                                     | 9 A.M.    | 0.169 c.c.                          |



III. *Buxus sempervirens* var. *arborescens*.—Specimens taken after similar exposure showed at first no assimilation, but after 3 hours at 15° C. weak and in 1 day fairly active from all parts with normal chlorophyll grains.

| 8 grammes green leaves.<br>(Twigs=1.7 grms.) |            |                                                    | 8 grammes living but browned leaves.<br>(Twigs=1.3 grms.) |            |                                                    |
|----------------------------------------------|------------|----------------------------------------------------|-----------------------------------------------------------|------------|----------------------------------------------------|
| Reading.                                     | Time.      | CO <sub>2</sub> evolved<br>per $\frac{1}{2}$ hour. | Reading.                                                  | Time.      | CO <sub>2</sub> evolved<br>per $\frac{1}{2}$ hour. |
| 180.0 c.c.                                   | 11 A.M.    |                                                    | 181 c.c.                                                  | 11 A.M.    |                                                    |
| 178.5 c.c.                                   | 11.30 „    | 1.5 c.c. }                                         | 179.5 c.c.                                                | 11.30 „    | 1.5 c.c. }                                         |
| 177.8 c.c.                                   | 12 „       | 0.7 c.c. }                                         | 178.8 c.c.                                                | 12 „       | 0.7 c.c. }                                         |
| 176.0 c.c.                                   | 2.30 P.M.  | 0.33 c.c. }                                        | 178 c.c.                                                  | 2.30 P.M.  | 0.16 c.c. }                                        |
| 175.4 c.c.                                   | 3 „        | 0.6 c.c. }                                         | 177.3 c.c.                                                | 3 „        | 0.6 c.c. }                                         |
| 174.6 c.c.                                   | 3.30 „     | 0.8 c.c. }                                         | 176.7 c.c.                                                | 3.30 „     | 0.6 c.c. }                                         |
| 173.1 c.c.                                   | 4.30 „     | 0.75 c.c. Light.                                   | 175.5 c.c.                                                | 4.30 „     | 0.6 c.c. Light.                                    |
| 172.5 c.c.                                   | 5 „        | 0.6 c.c. }                                         | 174.7 c.c.                                                | 5 „        | 0.8 c.c. }                                         |
| 171.8 c.c.                                   | 5.30 „     | 0.7 c.c. }                                         | 173.8 c.c.                                                | 5.30 „     | 0.9 c.c. }                                         |
|                                              | Next day.  |                                                    |                                                           | Next day.  |                                                    |
| 158.5 c.c.                                   | 9.30 A.M.  | 0.83 c.c. }                                        | 159.1 c.c.                                                | 9.30 A.M.  | 0.92 c.c. }                                        |
|                                              | 3rd day.   |                                                    |                                                           | 3rd day.   |                                                    |
| 157.7 c.c.                                   | 10.30 A.M. | 0.4 c.c. }                                         | 158.2 c.c.                                                | 10.30 A.M. | 0.45 c.c. }                                        |

In the case of *Buxus* it is possible that the large amount of CO<sub>2</sub> which the frozen leaves at first evolve when thawed is partly due to the setting free of pre-formed CO<sub>2</sub>, retained mechanically by the frozen tissue. Hence the subsequent fall followed by a secondary rise to the true respiratory maximum.

Without attaching too much importance to the results thus obtained, still it appears that (1) respiration may be active whilst assimilation is still absent, and (2) respiration recovers from exposure to a low temperature more rapidly and responds more readily to an increase of temperature than assimilation does.

In certain cases preparations which at 1° C. show a weak evolution of oxygen, when brought to a temperature of from 15° C. to 20° C. cease in a few minutes to evolve oxygen, after a short interval, which may be as long as an hour, recommencing to evolve oxygen with normal rapidity. This stoppage does not appear to be due, as at first seems to be the case, to the effect of



TABLE J.

|                            | 6 hours at 0°·0 C. to 0°·2 C.                                                                                                                                                                                                     | 1 day at 0°·0 C. to 0°·2 C.                                                                                                        | 2 days at 0°·0 C. to 0°·3 C.                                                                                                                                                                                                                                                                    | 5 days at 0°·5 C. to 1-2° C.                                                                                                                                                                         |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Chara fragilis.</i>     | No evol <sup>n</sup> of O. Slow rotation in most end cells. At 20° C. rotation soon rapid; evol <sup>n</sup> of O commences in 3 h., and is fairly active in 5 h.                                                                 | Apical and cortical cells dead. In 2 h. at 20° C. weak and in 4 h. fairly active evol <sup>n</sup> of O from many medullary cells. | Same as after 1 day.                                                                                                                                                                                                                                                                            | End and cortical cells dead. Most medullary cells living and show in 3 h. an active evol <sup>n</sup> of O.                                                                                          |
| <i>Elodea canadensis.</i>  | No evol <sup>n</sup> of O. Few cells, slow creeping rotation. At 20° C. rotation at once increases, in 5 minutes is fairly active. Assim. is fairly active in 10-15 minutes.                                                      | Rotation as before but evol <sup>n</sup> of O active after 3 h. at 20° C.                                                          | Leaves all living. Assim. as after 1 day. No rotation; slow in 15 minutes at 20° C., active in 3 h.                                                                                                                                                                                             | No rotation and no evol <sup>n</sup> of O. In 15 minutes at 20° C. slow rotation. In 3 h. weak evol <sup>n</sup> of O and active rotation. In 5 h. assim. is mod. active, and in 1 day quite active. |
| <i>Nitella flexilis.</i>   | 1 day at 0°·2 C. to 0°·5 C. rotation present but slow. No evol <sup>n</sup> of O. In 15 min. at 20° C. rotation active, but no evol <sup>n</sup> of O. Weak evol <sup>n</sup> of O. in ½ h., quite active in 2 h.                 |                                                                                                                                    | 4 days at 0°·5 C. to 1°·5 C. Nearly all cells living; large axial cells show slow rotation. In ¼ h. at 20° C. rotation active in axial cells, slow in rest; few cells show weak evol <sup>n</sup> of O. In 1 h. rotation normal, evol <sup>n</sup> of O fairly active, and quite active in 3 h. |                                                                                                                                                                                                      |
| <i>Cladophora</i> species? | After 1 day at 0° C. to 1° C. No evol <sup>n</sup> of O. Many cells dead, especially in younger branches. After ¼ h. at 20° C. weak evol <sup>n</sup> of O from older living branches, stronger in 1 h. and fairly active in 3 h. |                                                                                                                                    |                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                      |



the shock induced by the sudden change of temperature, causing a temporary stoppage of assimilation, for in normal actively assimilating preparations the evolution of oxygen is not perceptibly affected by sudden changes of temperature for short periods of time, provided that the range lies within the critical points. The phenomenon is probably simply due to respiration increasing more rapidly than and temporarily overpowering assimilation.

The main results obtained by experimenting with freshwater algæ, &c. are given below, and show that these plants are not very resistant to cold, all those examined being killed by being frozen.

TABLE K.

|                              | 3 days at 0°·5 C. to 2° C.                                                                                                                                                                                                                 | 3 days averaging 2° C.<br>(1° C. to 4° C.).                                                                                                                                                                                                         |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Edogonium</i><br>species? | Nearly all filaments living. No evol <sup>n</sup> of O, weak in 1 h. at 20° C, fairly active in 3 h., about normal in 5 h.                                                                                                                 | No evol <sup>n</sup> of O (at 1° C.); in $\frac{1}{2}$ h. at 20° C. weak and in 3 h. active.                                                                                                                                                        |
| <i>Spirogyra</i><br>species? | Most filaments dead, few living, plasmolysable and with normal Chl. bands, but these do not recover and soon die.                                                                                                                          | Most filaments living. No evol <sup>n</sup> of O (at 1° C.), in 1 h. at 20° C. weak, in 3 h. about normal assim., in all living cells.                                                                                                              |
| <i>Chara fragilis</i> .      | No assim. or rotation. Most end cells dead, nearly all cortical and medullary cells living. In 15 min. at 20° C. very weak evol <sup>n</sup> of O, but no rotation. Rotation commences in 1 h., and in 3 h. both it and assim. are active. | <i>Nitella flexilis</i> .—Slow rotation in older axial cells only. No evol <sup>n</sup> of O (at 1° C.). Evol <sup>n</sup> of O, shown first by the younger branches in which rotation is still slow. In 1 h. at 20° C. rotation and assim. active. |

With freshwater plants even after exposure reaching nearly to the critical point, no very marked after-effect is produced upon assimilation. This is probably because, owing to their slight powers of resistance to cold, the temperatures to which they can be exposed without being permanently injured are necessarily relatively high. It is very difficult, if not impossible, to cause even by very prolonged exposure to cold a stoppage of rotation in the larger axial cells of *Nitella* without their being permanently injuriously affected, and probably the same is the case with the



internodal medullary cells of *Chara*. In other cases, it is comparatively easy by exposure to cold to cause a stoppage of rotation. There does not seem to be any inherent essential connection between the recovery of rotation and the recovery of assimilation—the one may be active whilst the other is still weak or absent.

Experiments with more resistant land plants show that within certain limits a short exposure to a very low temperature produces the same result as a longer exposure to a higher temperature.

TABLE L.

|                                                                                                                                    |                                                                                                    |
|------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| Plants for 3 weeks at average temperature of $-2^{\circ}$ C. to $-3^{\circ}$ C.<br>Examined at $1^{\circ}$ C. All remained living. |                                                                                                    |
| <i>Ceratodon purpureum</i> ;<br><i>Oxalis Acetosella</i> .                                                                         | Weak evol <sup>n</sup> of Oxygen, which after 1 h. at $15^{\circ}$ C. is active.                   |
| <i>Bryum caespitium</i> ;<br><i>Sphagnum acutifolium</i> .                                                                         | No evol <sup>n</sup> of O; commences in $\frac{1}{4}$ h. at $15^{\circ}$ C. and is in 1 h. active. |
| <i>Selaginella helvetica</i> ;<br><i>Marchantia polymorpha</i> ;<br><i>Lunularia rediviva</i> .                                    | No evol <sup>n</sup> of O; in 1 h. at $15^{\circ}$ C. fairly active and in 3 h. quite active.      |

TABLE M.

|                                                                                                                                                                                                                                           |                                                                                                                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Plants for 15 hours at a temperature falling from $-2^{\circ}$ C. to $-9^{\circ}$ C.<br>Examined at $1^{\circ}$ C.                                                                                                                        |                                                                                                                                         |
| <i>Brassica Napus</i> .                                                                                                                                                                                                                   | Seedlings. Assim. faint or imperceptible; in 3 h. at $15^{\circ}$ C. active.                                                            |
| <i>Hedera Helix</i> ; <i>Cupressus sempervirens</i> .                                                                                                                                                                                     | No evol <sup>n</sup> of Oxygen, but in 3 h. at $15^{\circ}$ C. assim. is quite active.                                                  |
| <i>Buxus sempervirens</i> ;<br><i>Thuja orientalis</i> .                                                                                                                                                                                  | No evol <sup>n</sup> of Oxygen. In 3 h. weak and in 1 day fairly active assim. A few leaf-cells in <i>Buxus</i> die and do not recover. |
| The same 5 plants exposed for 6 h. to a temperature falling from $0^{\circ}$ C. to $-8^{\circ}$ C. showed when examined at $1^{\circ}$ C. a weak but distinct power of assimilation, which after 1-2 h. at $15^{\circ}$ C. became active. |                                                                                                                                         |

A series of experiments performed with plants from the temperate, cold, and tropical houses showed as regards the function of assimilation very different powers of resistance to exist between these three classes of plants. In Table N the leafy branches of cool temperate plants were exposed under

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⊖ ⊖ ⊖



TABLE N.

Plants from cool temperate house, average temperature 10° C.

|                              | For 6 hours at -4° C.<br>to -6° C.                                   | 14 hours at -2° C.<br>to -8° C.                                                             | 2nd day, temp. -6° C.<br>to -10° C.                                                                                    | 3rd day, temp. -5° C.<br>to -8° C.                                                                            |
|------------------------------|----------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| <i>Ilex latifolia</i> .      | No evol <sup>n</sup> of O. In 1 h. at 15° C. assim. is quite active. | Few cells killed and Chl. grains disorganized. No assim.; in 3 h. weak and in 1 day active. | Few leaf-cells living, plasmolysable, and with normal Chl. grains. No recovery, and in a few hours all cells are dead. | All leaf-cells dead.                                                                                          |
| <i>Euonymus japonicus</i> .  | Distinct but weak assim., which after 1 h. at 15° C. is active.      | As in <i>Ilex</i> , but assim. active in 3 h.                                               | As in <i>Ilex</i> .                                                                                                    | As in <i>Ilex</i> .                                                                                           |
| <i>Rhamnus Alaternus</i> .   | As in <i>Euonymus</i> .                                              | As in <i>Euonymus</i> .                                                                     | Many cells dead. No assim.; in 5 h. at 15° C. weak and in 1 day fairly active assim. in all living cells.              | Plenty of living leaf-cells. No assim.; feeble in 3-5 h., fairly active in 1 day.                             |
| <i>Prunus Laurocerasus</i> . | As in <i>Euonymus</i> .                                              | As in <i>Euonymus</i> .                                                                     | As in <i>Rhamnus</i> .                                                                                                 | Many cells dead. After 5 h. at 15° C. still no assim., in 1 day distinct but weak assim. in all living cells. |



TABLE O.

|                                                                                                     | 1 day. Temp. 0°·2 C.<br>to 1° C.                                                                              | 2 days. Temp. 0°·0 C.<br>to 1°·0 C.                                               | 3rd day. Temp. 0°·5 C.<br>to 1°·5 C.                                            | 5th day. Temp. 0° C.<br>to 1° C.                                                                                                                  |
|-----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Bambusa arundinacea</i> ;<br><i>Hibiscus Rosa-sinensis</i> ;<br><i>Tradescantia geniculata</i> . | Distinct but weak assim., which in $\frac{1}{4}$ h. at 15° C. is active.                                      | Weak evol <sup>n</sup> of O, which in $\frac{1}{2}$ h. at 15° C. is quite active. | Very weak evol <sup>n</sup> of O, which is in 1 h. at 15° C. active.            | No evol <sup>n</sup> of O, none after 2 h. at 6-8° C., then at 15° C. in $\frac{1}{4}$ h. weak evol <sup>n</sup> of O, which in 1-3 h. is active. |
| <i>Buddleia diversifolia</i> .                                                                      | No evol <sup>n</sup> of O, but at 15° C. assim. commences in a few minutes, and is active in $\frac{1}{2}$ h. | No evol <sup>n</sup> of O, but in 1 h. at 15° C. active assim.                    | .....                                                                           | No evol <sup>n</sup> of O. After 2 h. at 6-8° C. weak, and at 15° C. is then in 1 h. active.                                                      |
| <i>Alpinia officinarum</i> .                                                                        | As in <i>Buddleia</i> .                                                                                       | As in <i>Buddleia</i> .                                                           | .....                                                                           | No evol <sup>n</sup> of O. After 2 h. at 6-8° C. still none, then at 15° C. in $\frac{1}{2}$ h. faint, in 1 h. fairly, and in 3 h. quite active.  |
| <i>Ficus parietalis</i> .                                                                           | As in <i>Bambusa</i> .                                                                                        | Weak evol <sup>n</sup> of O, which in $\frac{1}{4}$ h. at 15° C. is active.       | No evol <sup>n</sup> of O. At 15° C. in 1 hr. fairly, and in 2 h. quite active. | As in <i>Alpinia</i> .                                                                                                                            |



TABLE P.

Tropical plants from hot-house, temperature 15° C. to 25° C., never below 10° C.

|                                 | At 1° C.                                                                                                                                        | At 1° C. for 4 hours, and then at 20° C.                                                 | At 5° C.                                                                                                                          | At 10° C.                                                   |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
| <i>Epidendrum floribundum</i> . | Evol <sup>n</sup> of O ceases in $\frac{1}{4}$ to $\frac{1}{2}$ h. At 20° C. at once recommences and is in 10 to 15 minutes active again.       | Evol <sup>n</sup> of O recommences in $\frac{1}{4}$ h. and is active in $\frac{1}{2}$ h. | Evol <sup>n</sup> of O ceases in an hour, at 20° C. at once recommencing.                                                         | After 5 h. evol <sup>n</sup> of O still quite active.       |
| <i>Aspidium violascens</i> .    | In 1 h. evol <sup>n</sup> of O very weak, in 4 h. none. At 20° C. is in $\frac{1}{2}$ h. active again.                                          | Weak evol <sup>n</sup> of O in $\frac{1}{4}$ h., in $\frac{1}{2}$ h. fairly active.      | After 4 h. weak evol <sup>n</sup> of O still present, which at 20° C. in $\frac{1}{4}$ h. is active.                              | Evol <sup>n</sup> of O remains active.                      |
| * <i>Mimosa pudica</i> .        | Evol <sup>n</sup> of O ceases in $\frac{1}{2}$ h., at 20° C. at once recommencing.                                                              | Weak evol <sup>n</sup> of O in $\frac{1}{2}$ h., in 1 h. fairly active.                  | Evol <sup>n</sup> of O ceases in 1 h. At 8° C. in 4 h.                                                                            | Evol <sup>n</sup> of O persists, but is weaker than normal. |
|                                 | After 1 day at 1° C.                                                                                                                            |                                                                                          | After 1 week at an average of 1° C. (0° C. to 2° C.).                                                                             |                                                             |
| <i>Aspidium violascens</i> .    | No evol <sup>n</sup> of O, in $\frac{1}{2}$ h. at 20° C. faint traces, in 1 h. moderately active, and in 2 h. quite active.                     |                                                                                          | No evol <sup>n</sup> of O, faint in 3 h. at 20° C., stronger in 5 h., fairly active in 1 day.                                     |                                                             |
| <i>Epidendrum floribundum</i> . | No evol <sup>n</sup> of O, in $\frac{1}{4}$ h. at 20° C. just perceptible, in $\frac{1}{2}$ h. weak, and in 1 h. active evol <sup>n</sup> of O. |                                                                                          | Many leaves with black dead patches. No evol <sup>n</sup> of O, none after 5 h. at 20° C., weak in 12 h., fairly active in 1 day. |                                                             |

\* Since in *Mimosa* there is neither a temporary nor a permanent stoppage of assimilation as the result of section-cutting, it follows that there is no direct connection between Assimilation and Irritability, a maximal excitation of the latter exercising no influence upon the former. In the drooping position of the leaves the actual amount of assimilation of the plant as a whole is certainly much lessened, but only because the position thereby assumed is, for physical reasons, less advantageous for assimilation, the inherent power of assimilation remaining unaffected. Thus, leaflets of *Mimosa pudica* and *Oxalis Acetosella* are found, when examined at night in the nyctitropic position, to be capable, on sudden exposure to light, of as active assimilation as when in the expanded diurnal position.



metal covers during a period of severe frost, the range of temperature being taken by a differential thermometer, after exposure the plants being thawed and examined at 1° C.

Leafy branches taken from the warm house (average temperature 10° C. to 20° C.) were kept in a glass case surrounded by snow, examined at 1° C. and then brought to a temperature of 15° C. (Table O).

With tropical plants assimilation ceases to be perceptible at a temperature several degrees above zero (Table P).

Experiments were also made with three-weeks old seedlings of certain plants developed under precisely similar conditions with regard to temperature (30° C.), exposure to light, amount of moisture, &c.

TABLE Q.

|                                                     | At -2° C. to -1° C. for 1 day, and then examined at 1° C.                                                                                       | 3 days at -1° C. to 1° C., then 1 day at -3° C. to -5° C.                                                                                                               |
|-----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Vicia Faba</i> ;<br><i>Helianthus annuus</i> .   | All leaves living. Very faint evol <sup>n</sup> of O at 1° C. In 1 h. at 20° C. fairly, and in 2-3 h. quite active evol <sup>n</sup> of Oxygen. | Almost all leaves and leaf-cells living. At 1° C. at first no evol <sup>n</sup> of O, in ½ hr. at 20° C. moderately, and in 1-2 h. quite active evol <sup>n</sup> of O. |
| <i>Ricinus communis</i> ;<br><i>Lupinus albus</i> . | All leaves living. At first no evol <sup>n</sup> of O. In 1 h. at 20° C. fairly, and in 2-3 h. quite active assim.                              | At -1° C. to +1° C. for 1 day.                                                                                                                                          |
| <i>Zea Mays</i> ;<br><i>Phaseolus vulgaris</i> .    | Few leaves killed, rest show no evol <sup>n</sup> of O, none after 2 h. at 20° C., weak in 3 h., fairly active in 5 h., normal in 1 day.        | No evol <sup>n</sup> of O at 1° C., but assim. returns rapidly at 20° C. and is normal in 1 hr. and all leaves remain living.                                           |

The above table clearly shows that the most important factor in determining the resistant power of any given plant to cold is the hereditary nature and disposition of the plant itself, but how this acts and what the exact relation between the two may be is impossible to say.

Though death caused by freezing is due to the withdrawal of water from the cell to form ice crystals, nevertheless resistance to desiccation and resistance to freezing are not necessarily



coincident. All plants which can withstand desiccation can also withstand freezing; but it is only plants which are very intolerant of, and very readily damaged by, a loss of water that are easily killed by freezing at a few degrees below zero. To very low temperatures, since the withdrawal of water is here complete, only those plants are resistant which are capable of withstanding complete desiccation\*.

The latent period of return of assimilation after exposure to cold is nearly always of relatively short duration, and is possibly produced by the withdrawal of water from the protoplasm, and represents the time necessary for the assimilatory plasma to completely return to its normal condition of metabolic activity.

As regards the temperature at which assimilation commences, Cloez and Gratiolet† found that it began in *Potamogeton Ceratophyllum* and *Conferva* between 10° C. and 15° C., and in *Vallisneria* above 6° C. With larch-leaves Boussingault‡ detected an evolution of oxygen at 0°·5 C. to 2°·5 C., and with meadow grasses at 1°·5 C. to 3°·5 C.; whilst Heinrich§ found that with *Hottonia palustris* bubbles of oxygen were first given off at 2°·7 C. By the more delicate Bacterium method an evolution of oxygen can be detected at temperatures several degrees lower than the above. It appears that all evolution of oxygen ceases in tropical plants between 4° C. and 8° C., in warm temperate, subtropical, and water-plants between 0° C. and 2° C., whilst in cool temperate, arctic, and alpine plants assimilation only ceases when the plants are frozen, *i. e.* at a few degrees below 0° C. In this last case the stoppage of assimilation is of purely physical origin, and is due to the withdrawal of water from the protoplasm to form ice crystals, leaving the assimilatory plasma in what is virtually a more or less completely desiccated condition.

In many cases freezing and the stoppage of assimilation are coincident with a cessation of vitality, but in others the plants may be completely frozen and assimilation stopped without the power of recovery being lost. In such plants both respiration and assimilation may function at zero, or even, and especially the former, a few degrees below it. Thus Clausen finds that

\* See Pfeffer, 'Physiologie,' Bd. ii. Cap. 10.

† Cloez and Gratiolet, in Journ. univ. Sc. France et à l'étranger, 30 Oct., 1850, No. 878.

‡ Boussingault, in Ann. Sc. nat. sér. v. t. x. (1869) p. 336.

§ Heinrich, in Landw. Versuchsst. xiii. 1871, p. 136.



cells of seedlings of Lupines only freeze at  $-4^{\circ}\text{C.}$ , and that hence above this temperature respiration can still continue. At zero he finds respiration to take place with  $\frac{1}{10}$ th to  $\frac{1}{15}$ th its optimal activity. Jumelle finds that with Conifers and Lichens respiration may not entirely cease till a temperature of  $-10^{\circ}\text{C.}$  is reached. This statement is rendered more probable by the researches of Dixon and Joly \*, who find that in a plant-tissue all the intracellular and imbibed water may not freeze until the temperature is lowered to  $-10^{\circ}\text{C.}$  or  $-12^{\circ}\text{C.}$  As regards assimilation, Jumelle states that *Evernia Prunastri*, *Picea excelsa*, and *Juniperus communis* at from  $-30^{\circ}\text{C.}$  to  $-40^{\circ}\text{C.}$ , if exposed to light, evolve oxygen and absorb  $\text{CO}_2$ . He supposes that the heat-rays which fall upon the specimens along with the light-rays when exposed to light may prevent all the water of the plant-cells being frozen even when exposed to from  $-30^{\circ}\text{C.}$  to  $-40^{\circ}\text{C.}$ , and thus permit of assimilation taking place, though in darkness all the contained water is frozen and respiration ceases at  $-10^{\circ}\text{C.}$  To this hypothesis two objections may be made:—(1) the same heating-effect would be produced upon the registering thermometer as upon the exposed specimens; and (2) if such a special heating-effect were produced, then the experiments would be valueless, for the specimens would be exposed to a temperature higher than that registered by the thermometer.

It is possible that the changes in the composition of the air of the receiver observed by Jumelle at these low temperatures may be due not to the continuance of any actual assimilation, but to physical diffusion, carbonic acid being slowly absorbed and oxygen slowly exhaled from the frozen specimens. The following facts make probable this assumption, thus:—(1) The amounts of gas absorbed and exhaled are relatively small. (2) The volumes of oxygen and  $\text{CO}_2$  which are exhaled and absorbed do not at all correspond. (3) The gaseous interchanges from the first diminish in amount as the exposure continues. (4) The gaseous interchanges do not alter in amount within very wide ranges of such low temperatures, and only increase when the temperature is raised to a few degrees below zero.

When the experiment is started, the plants being exposed to light, and at first at a temperature above zero, will continue to assimilate until the temperature falls below zero and the plants

\* "The Path of the Transpiration-current," in *Annals of Botany*, 1895.



are completely frozen. The intracellular and extracellular gases which the frozen tissues of the specimens now hold will contain a much larger percentage of oxygen, and a much smaller percentage of  $\text{CO}_2$ , than the assimilatory mixture of air and  $\text{CO}_2$  contained in the receiver in which the plant is lying. Owing to the frozen condition of the tissues, this inequality is only slowly adjusted by diffusion to and from the surrounding air contained in the receiver, the result being that the air of the receiver will lose  $\text{CO}_2$  and gain oxygen just as if slight assimilation had taken place.

Besides the above, there are very weighty *à priori* reasons for doubting that any assimilation could possibly take place at a temperature of from  $-30^\circ \text{C.}$  to  $-40^\circ \text{C.}$  As we have seen throughout, assimilation seems to be a function which remains active through much narrower ranges of temperature than respiration does. Thus the minimal temperature for respiration is apparently, as a general rule, several degrees lower than that for assimilation, whilst the maximal temperature for respiration is from  $5^\circ \text{C.}$  to  $15^\circ \text{C.}$  above that for assimilation, and the same general differences appear to hold good for all plants, a difference in the minimal and maximal points for respiration being accompanied by a corresponding difference in those for assimilation.

#### *Effect of Irrespirable Gases upon Assimilation.*

Direct observations were first made under the microscope in a gas-chamber with a hanging drop and *Bacterium Termo*, mosses and water-plants being examined (*Bryum caespititium*, *Orthotrichum affine*, *Dicranum scoparium*, *Elodea canadensis*, and *Chara fragilis*). In an atmosphere of  $\text{H}$ , the preparations continue to evolve oxygen for as long as they are exposed to light (10 to 12 hours), the necessary  $\text{CO}_2$  for assimilation being supplied by the Bacteria in the hanging drop. At the end of this time the evolution of oxygen is with moss-leaves still quite active, but with *Chara* and *Elodea* has, as a general rule, become distinctly less active than it was at first.

If preparations of *Chara* or *Elodea* are kept in a stream of  $\text{H}$ , either in light or in darkness, they finally in from a few to many hours lose the power of assimilating and evolving oxygen, though they may still continue to show rotation. Even if kept exposed to light in a current of an assimilatory mixture



of H and CO<sub>2</sub>, the preparations also finally lose the power of assimilation.

Moss-leaves, however, under these conditions retain the power of assimilation for an indefinite length of time. The explanation is that, in the first two plants under these abnormal conditions, a state of assimilatory inhibition is induced in the exposed specimens, from which at first recovery is possible if the plants are brought under normal conditions again, *i. e.* on exposure to air after a longer or shorter latent period the power of assimilation returns. If the exposure has been too prolonged, the power of recovery is lost and the preparations finally die.

If green plants are exposed to light in an atmosphere of H, at the end of several hours' exposure the surrounding gas is found to have undergone no perceptible change in composition, but nevertheless during the whole time of the exposure both assimilation and respiration are going on, and the specimens are continuously evolving traces of both oxygen and CO<sub>2</sub>, which may be detected by the Bacterium method and by Phenolphthalein respectively.

In an atmosphere of pure CO<sub>2</sub> in all cases, assimilation and evolution of oxygen cease almost immediately (in 1 to 2 minutes). If the CO<sub>2</sub> is now replaced by H, a normal evolution of oxygen is at once shown, but if the preparations are kept in an atmosphere of CO<sub>2</sub> for some time they may only recommence to evolve oxygen and assimilate after a longer or shorter period of time has elapsed. In an atmosphere of CO<sub>2</sub> moss plants may remain living for many days or even weeks, but *Chara* or *Elodea* cease to show rotation after 15 to 20 minutes or 5 to 10 minutes respectively, and after 2 or 3 hours' immersion are killed; whereas in an atmosphere of pure hydrogen *Chara* may remain living for half a day (12 hours). In an atmosphere of hydrogen rotation gradually becomes less and less rapid, but when suddenly surrounded by CO<sub>2</sub> rotation undergoes an immediate diminution to as much as one half its previous velocity, the difference being due to the fact that in the latter case, besides the removal of the necessary oxygen, a direct and immediate poisonous and retarding influence is produced.

Mosses, however, as well as many plants of lower organization, are remarkably resistant not only to the asphyxiating effects of immersion in an atmosphere of hydrogen, but also to the



poisonous action of an atmosphere of  $\text{CO}_2$ . Specimens of the above three mosses were kept in moist atmospheres of hydrogen and  $\text{CO}_2$  at a temperature of from  $15^\circ \text{C.}$  to  $20^\circ \text{C.}$  in the darkness for long periods of time, and then examined.

The mosses are kept moist in a suspended tray within a graduated bottle which is filled with water. Gas is driven in, driving out water through the exit-tube the shorter distal end of which dips under mercury and leaving the receiver filled with the gas in question. In the same way, a mixture of gas with air or oxygen in any given proportion can be readily introduced and when necessary, by first siphoning or driving water backwards and then driving it out again, fresh gas can be introduced without an exposure of the plants to the air being necessitated.

TABLE R.

|                                 | 1 week in darkness in<br>atmosphere of H.                                                                                                                                                                    | 4 weeks in darkness in<br>75 p.c. $\text{CO}_2$ + 25 p.c. air.                                                                                                                                     |
|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Bryum<br/>caespititium.</i>  | Nearly all leaves and leaf-cells living and normal. Assimilation in a few leaves weak, but in rest active.                                                                                                   | Most leaves and leaf-cells living. In a few leaves assim. is very weak, but in rest is active.                                                                                                     |
| <i>Orthotrichum<br/>affine.</i> | Many older leaves and leaf-cells dead. Living leaves assim. absent or in parts only. Apical young leaves assim. is mod. active. In 1 day in D. Ch. in air, assim. active in most cases and in 2 days in all. | As in <i>Bryum</i> .                                                                                                                                                                               |
| <i>Dicranum<br/>scoparium.</i>  | Only apical leaves living, wholly or partly. No assim. In 1 day weak assim. in few leaves, in 2 days weak in all leaves, active in some, and in 4 days active assim. in all living leaves.                   | Most leaves and leaf-cells living. Weak assim. in young leaves, in older leaves none. In 1 day assim. in all living cells, which in 2-3 days is normal and starved appearance of cells disappears. |

For comparative purposes, plants were also kept for 4 weeks in darkness in moist air at  $15^\circ \text{C.}$  On examination, the leaves of these were found to be almost all living and showed fairly active or in a few cases only weak assimilation. The chlorophyll grains are, beyond having a slight yellowish tinge and being devoid of starch, normal, whilst the protoplasm is clear, vacuolated, and



transparent, and in *Dicranum* without oil-drops. After 2 or 3 days' exposure to light, the plants are quite normal in all respects. The slight yellowish tinge acquired by the chlorophyll grains and the starvation phenomena exhibited are therefore results of the absence of light.

Table S (p. 407) gives the results of keeping the mosses for long periods in darkness in an atmosphere of  $\text{CO}_2$ , temperature averaging  $15^\circ \text{C}$ .

It is worthy of remark that in many cases, after prolonged exposure, cells which show no assimilation do not recover that function even though they may remain living and plasmolysable for 2 or 3 days. Such cells have permanently lost the power of assimilation and are, as long as they live, nourished as a non-chlorophyllaceous cell is, though to all outward appearance the chlorophyll-apparatus is normal and intact. Attempts were made to keep such cells living by supplying them with artificial nutrient media, but without success. Apparently when asphyxiation produces a permanent stoppage of assimilation, it involves internal changes exercising so profound an influence upon the vitality of the cell that sooner or later death follows as a necessary consequence.

In *Dicranum* the sequence of changes from life to death are :— The protoplasm gradually becomes more opaque, granular, and greyish, and also less readily plasmolysable, assimilation becomes weaker and weaker and finally stops; in this condition the cell may remain with the chlorophyll grains green and unaltered for a few days; in the first stages assimilation can return and the cell become normal again, but in the later stages the power of recovery is totally lost, and this is finally followed by the shrinking of the protoplasm from the cell-wall, the chlorophyll grains turning first yellowish and then rapidly losing all colour, until finally the cell-cavity is incompletely occupied by a greyish, rather opaque, but more refractive than when living, granular amorphous mass in which neither colour nor chlorophyll grains can be distinguished.

Boussingault\* first noticed the phenomena of "asphyxie" and found by means of the gas-analytical method that with Phanerogams in  $\text{H}$ ,  $\text{CH}_4$ ,  $\text{N}$ , or  $\text{CO}_2$  asphyxiation takes place and

\* Boussingault, 'Agronomie, Chimie Agricole et Physiologie,' 1868, iv. p. 329.



TABLE S.

|                             | In CO <sub>2</sub> for 1 week at 15° C.                                                                              | In CO <sub>2</sub> for 2 weeks.                                                                                                                                                       | In CO <sub>2</sub> for 4 weeks.                                                                                                                                                                                                       |
|-----------------------------|----------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Bryum caespitium.</i>    | Many leaves dead, but rest all or many cells living and showing active assimilation.                                 | Most leaves and leaf-cells living. Assimilation fairly active, but in many cases weak and in few leaves imperceptible. In 1 day normal assimilation in all living leaves.             | Apical tufts mostly living. No evolution of oxygen. In 4 hours weak assimilation in many cells, in most still none. In 1 day many cells die, but rest show weak or moderately active assimilation, and in 2 days active in all cases. |
| <i>Orthotrichum affine.</i> | Younger leaves living, but assimilation weak or imperceptible. In 2 hours assimilation stronger and in 1 day normal. | Younger leaves living, a few show weak assimilation, mostly none. In 3-4 hours weak assimilation in most leaves, in 1 day fairly active.                                              | Younger leaves, more or less cells living, but no assimilation. In 4 hours most living cells show weak assimilation, which in 1 day is fairly active.                                                                                 |
| <i>Dicranum scoparium.</i>  | Most leaves living. Assimilation weak to moderately active, and in 1 to 2 hours about normal.                        | Upper leaves mostly living. Assimilation absent, but in inner leaves of apical tuft assimilation weak to active, and in 3-4 hours normal. In rest normal assimilation in 1 to 2 days. | Younger leaves, more or less cells living. No assimilation, in 1 day assimilation still weak or absent. In 3-4 days few apical tufts still living and these show active assimilation.                                                 |



assimilation is first weakened and then permanently stopped. Even with mosses which are very resistant to asphyxiation similar results are finally produced, and, as we have seen above, the stoppage of assimilation is not necessarily permanent, but instead recovery may take place if the plants are brought under normal conditions again.

*Effects of Ether upon Assimilation.*

F. Schwarz \*, working with the bubble-counting method, found that in a saturated watery solution of ether water-plants cease in a few minutes to assimilate and no recovery takes place, the specimens having been fatally affected. Similarly, Pringsheim †, using the Bacterium method, found that anæsthetics such as chloroform and ether stop assimilation, but the cells cannot be restored to life and the chlorophyll apparatus is changed and altered. Bonnier and Mangin ‡, using the gas-analytical method, found that with certain Phanerogams, by using a measured quantity of ether, assimilation may be stopped whilst respiration continues unchecked, thus confirming the results obtained by Claude Bernard with chloroformed water-plants ('Leçons sur les phénomènes de la vie,' 1878, p. 278).

In the following experiments with Mosses, the plants were kept in an atmosphere containing ether vapour for varying periods of time. After being kept for 30 minutes and for 1 hour under a bell-jar over a saturated solution of ether in water (10 parts of water to 1 part ether) and then examined in the usual method, the three mosses experimented with gave the results shown in Table T. Care must be taken that no appreciable amount of ether remains adhering to the leaves, as a very slight amount of ether is sufficient to inhibit the movement of *B. Termo*. A few seconds' exposure to air is, however, sufficient to allow of the evaporation of all the ether from the leaves examined.

\* F. Schwarz, "Zur Kritik der Methode des Gasblasenzählens an submersen Wasserpflanzen," in Bot. Untersuch. v. Tübingen, 1881, p. 102.

† Pringsheim, "Ueber die Abhängigkeit der Assimilation grüner Zellen von ihrer Sauerstoffathmung," in Sitzungsberichte der Akademie der Wissenschaften zu Berlin, 1887.

‡ Bonnier and Mangin, in Ann. Sc. nat. sér. vii. t. iii. 1886, p. 14.



TABLE T.

|                             | After 30 minutes' exposure.                                                                                                                                                                                                                | After 1 hour's exposure.                                                                                                                                                                                                                           |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Bryum cespitium.</i>     | Few leaves, and leaf-cells dead, but rest living and assimilating actively.                                                                                                                                                                | Most leaves and leaf-cells living, and in all of these assimilation is active.                                                                                                                                                                     |
| <i>Orthotrichum affine.</i> | Most leaves and leaf-cells living and assimilation active, but a few leaves with plenty of living leaf-cells show no perceptible or extremely weak assimilation. In 2-3 h. many of these cells die, but the rest show normal assimilation. | Many leaves and leaf-cells dead and in living cells assimilation faint or imperceptible. In 5 h. rather weak, and in 1 day active assimilation in all living leaves.                                                                               |
| <i>Dicranum scoparium.</i>  | As in <i>Orthotrichum</i> .                                                                                                                                                                                                                | Most leaves and leaf-cells living and assimilation fairly active. Few leaves with all or many cells living, no assimilation. Many of these die in from a few hours to a day, but in rest assimilation returns and is normal in from 5 h. to 1 day. |

Using strong solutions of ether and short exposures the stoppage of assimilation seems, especially with *Bryum*, to be almost immediately followed by the death of the cell, and hence in such cases it is difficult to find living but non-assimilating cells. A series of more prolonged exposures were therefore made, the same plants being placed under bell-jars over a given quantity of a  $\frac{1}{10}$  saturated watery solution of ether (1 part ether to 100  $H_2O$ ), the resulting atmosphere containing 1 volume of ether to from 15 to 30 of air, and hence sufficient to burn or form an explosive mixture. During prolonged exposures the atmosphere is renewed from time to time and fresh ether solution introduced.

Table U shows clearly the pronounced resistant power exhibited by Mosses to prolonged exposure to dilute ether-vapour, as well as the marked stoppage of assimilation which finally occurs. In *Dicranum* one result of prolonged exposure is that the protoplasm in many cases becomes darker, more greyish and opaque than normal; but this peculiarity is exhibited by cells in which assimilation is still present, and may remain for some time in cells to which the power of assimilation has returned.



TABLE U.

|                             | After 18 hours' exposure.                                                                                                                                                                                                        | After 3 days.                                                                                                                                                                                                            | After 6 days.                                                                                                                                                                                                                |
|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Dicranum scoparium.</i>  | Most leaves and leaf-cells living. Weak assim. in leaves of apical tuft, older leaves none, but in 2-3 h. weak assim. After 1 day in D. Ch. few living leaves, still no assim, but in 2 days normal assim. in all living leaves. | Apical leaves living but assim. weak; older leaves, though plenty of leaf-cells living no assim. In 1 day most living leaves assim. fairly active, few still not assimilating are by 2nd day dead; rest assim. normally. | Upper leaves more or less leaf-cells living but no assim. In from 2-3 h. weak assim. in most living leaves, which after 1 day is active in the upper leaves, and in 2-3 days all living leaves show about normal. assim.     |
| <i>Bryum caespitium.</i>    | Almost all leaves and leaf-cells living and assim. active.                                                                                                                                                                       | Most leaves and leaf-cells living. In most apical leaves assim. fairly active, but in rest assim. weak or absent. In 1 day normal assim. in all living leaves.                                                           | Many plants with more or less leaves and leaf-cells living. Few young leaves show weak assim., rest none. In 1 day assim. fairly active, but few living leaves still assim. In 2-3 days all living leaves show normal assim. |
| <i>Orthotrichum affine.</i> | Almost all leaves and leaf-cells living, but assim. in most cases weak, but in 2-3 h. is normal.                                                                                                                                 | Almost all leaves living and showing weak assim., but in few cases none. In 1 day normal assim. in young leaves, and in 2 days in all cases.                                                                             | Younger leaves weak assim., older leaves none. In 2-3 h. assim. in younger leaves active. In 1 day weak assim. in older living leaves, and in 2-3 days normal assim. in all cases.                                           |



It is not therefore concomitant with any loss of assimilatory power. In many cases the leaf-cells of *Dicranum*, especially at the bases of the leaves, exhibit a marked longitudinal striation, somewhat resembling that shown by a voluntary-muscle fibre. As before, the presence of this striation is quite independent of the presence or absence of assimilation, and disappears in a day or two if the cells remain living. In all cases the chlorophyll grains remain normal and unaltered whether assimilation be present or absent so long as the cell remains living.

*Effects of Treatment with Acid upon Assimilation.*

The acid employed was phosphoric acid, a 1-per-cent. solution being made by adding to 1 c.c. of a thick sirupy solution of  $\text{H}_3\text{PO}_4$ , 99 c.c. of water. A very dilute acid solution suffices to inhibit the movement of *B. Termo*. Thus in a 0.001 per cent. solution the movement continues apparently unaffected, in a 0.005 per cent. solution it remains but is not so active, in a 0.01 per cent. solution it slows and stops in a few minutes.

If preparations of end cells of *Chara* be made in a few drops of 0.01 per cent.  $\text{H}_3\text{PO}_4$  in closed cells with Bacteria, rotation is soon slowed and in half an hour has in most cells ceased, though in others it is fairly active. As rotation ceases it undergoes temporary increases, attaining almost normal rapidity for a few minutes or so and then slowing again. These increases become shorter and less frequent, and finally the rotation comes to a standstill. In half an hour the Bacteria, which were at first motionless, begin to move, at first slowly whenever oxygen is present, and later more rapidly as the acid is absorbed by the *Chara* filaments. The cells in which rotation is still present show a weak but distinct evolution of oxygen. In about 2 hours rotation has stopped in all cases, but the evolution of oxygen does not cease for another 30 to 60 minutes; and now the power of recovery is completely lost, *i. e.*, with short exposure to, for *Chara*, relatively strongly acid solutions rotation ceases before the evolution of oxygen does, and when the power of assimilation ceases to be manifested, recovery is no longer possible.

Plants of *Chara* are fatally affected by immersion for several hours in a 0.001 per cent. solution of  $\text{H}_3\text{PO}_4$ , provided that a sufficiently large quantity of the acid solution is employed and that the specimens are quite free from chalk. *Chara* is excessively sensitive to immersion in acid media, and far more so



than other water-plants are. The chalky incrustation on *Chara* is certainly most important as a protection against the injurious action of even minute traces of fixed acid.

In plants of *Chara fragilis* after 16 hours' immersion in 0.0005 per cent.  $H_3PO_4$  all end cells and most cortical cells are dead, no evolution of oxygen is shown, and none after 2 hours in water. In 4 hours a weak evolution of oxygen in parts and in some cells rotation can be seen. Next day all living parts show moderately active assimilation. In a few cases the cortical cells are killed and become white, blanched, and transparent, whilst the medullary cell, now rendered plainly visible, shows active rotation and assimilation.

The following tables give the results of experiments with water-plants and mosses, the plants being completely immersed in solutions of varying strengths for many hours and then examined after washing to remove all traces of adhering acid.

TABLE V.

|                                          | <i>Elodea canadensis.</i>                                                                                                                                                                                                                                                                                                        | <i>Utricularia vulgaris.</i>                                                                                                                                                                                                                                         |
|------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| After 24 hours in 0.01 p. c. $H_3PO_4$ . | At first no rotation, but in a few minutes it commences and is in $\frac{1}{4}$ – $\frac{1}{2}$ hour active. Most leaves no assim., but weak evol <sup>n</sup> of O in many, and in $\frac{1}{2}$ h. weak evol <sup>n</sup> of O in nearly all cases, in 1 h. in all cases and active in some. In 2–3 h. assim. is about normal. | Most branches weak evol <sup>n</sup> of O, active in few, and in few none. In $\frac{1}{2}$ h. weak evol <sup>n</sup> of O in all cases, in many branches active, and in 1 to 2 h. about normal assim. in all cases.                                                 |
| After 24 hours in 0.02 p. c. $H_3PO_4$ . | Central leaves of apical tufts and basal cells of most leaves living, but no evol <sup>n</sup> of O. After 4 h. weak in many cases, after 8 h. in all cases, and in 1 day active evol <sup>n</sup> of O from all living cells.                                                                                                   | Younger branches living, but no evol <sup>n</sup> of O. In 2 h. weak evol <sup>n</sup> of O in some branches and in 4 h. in most. In 8 h. assim. is moderately active, but in few branches still weak or absent. In 1 day active assim. in all cases.                |
| After 24 hours in 0.04 p. c. $H_3PO_4$ . | Leaves of apical tuft more or less cells living, but no evol <sup>n</sup> of O, none after 2 h., weak in few cases in 4 h.; in 8 h. weak assim. in most living cells, which in 1 day is moderately active.                                                                                                                       | Young adult branches partially or entirely living. No evol <sup>n</sup> of O and none after 2 h. Weak in 4 h. from most living branches, in 8 h. in few cases mod. active, but in some still none. In 1 day all living branches show from weak to mod. active assim. |



TABLE W.

|                             | 1 day in 0.1 p. c. $H_3PO_4$ .                                                     | After 2 days in 0.1 p. c. $H_3PO_4$ .                                                                                                                                                                         |
|-----------------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Bryum caespitium.</i>    | Plants almost unaffected.                                                          | In most leaves no evol <sup>n</sup> of O from apical half, and here more or less cells may be dead. In rest active assim. In 1 day fairly active assim. in all cases.                                         |
| <i>Catharinea undulata.</i> | As in <i>Bryum</i> .                                                               | Few leaves active, evol <sup>n</sup> of O most weak, and all cells living; in many assim. absent, and here more or less cells dead. After 1 day weak evol <sup>n</sup> of O in latter case, active in former. |
| <i>Dicranum scoparium.</i>  | Nearly all leaves living. No assim. In 1 day weak or absent, but in 2 days active. | Most leaves and leaf-cells living. No assim. After 1 day assim. still weak or imperceptible. In 2-3 days several leaves die but in rest assim. is active.                                                     |

It will be noticed that the mosses are very much more resistant than water-plants are; and though this is undoubtedly partly due to an inherent difference of vitality it is also certainly partly due to the fact that the acid solution penetrates with greater difficulty through the cell-walls of mosses than through those of water-plants. Pfeffer\* has shown that the acid penetrates both cell-wall and protoplasm and appears in the cell-sap. The death of a cell immersed in an acid solution is soon followed by the contraction of the cell-contents and a rapid disorganization and decolorization of the chlorophyll grains.

*Effect of Alkali upon Assimilation.*

Darwin†, in 1882, noticed that treatment with dilute alkalies caused in many cases in the living cell a precipitate to be formed in the cell-sap, and that only after prolonged exposure in very dilute solution the cells are killed. The precipitate consists, as has been conclusively shown by Pfeffer‡ and Klein§,

\* Pfeffer, "Ueber Aufnahme von Anilinfarben in lebenden Zellen," in *Untersuch. aus dem Bot. Inst. zu Tübingen*, p. 290.

† Darwin, *Insectivorous Plants*, 1882.

‡ Pfeffer, *loc. cit.* (Ueber Aufnahme von Anilinfarben), p. 240.

§ Klein, in *Flora*, 1892, p. 400; in *Ber. d. Bot. Gesell.* 1892, p. 237; in *Bot. Centralblatt*, 1894, Bd. lvii. p. 187.



of tannin and other substances which the penetrating ammonium carbonate causes to be excreted. The phenomenon is therefore a proof that the alkali penetrates both the cell-wall and the lining protoplasm.

For experiments upon assimilation a 0·1-per-cent. solution of ammonium carbonate (0·1 gramme of salt to 100 grammes  $H_2O$ ) was employed. In this strength of solution the movement of *B. Termo* remains active as long as oxygen is present, and hence the specimens can be examined after immersion in a 0·1-per-cent. without being washed or can be examined directly in it. To see whether recovery is possible or not it is of course necessary to remove all alkali by washing in either fresh water or water containing a trace (0·001 per cent.  $H_3PO_4$ ) of acid.

*Chara fragilis* is killed by 16 hours' immersion in a 0·1-per-cent. solution, but after 10 hours' immersion, though most end and cortical cells are dead, a few are living and normal in appearance, but show neither rotation nor assimilation. After washing, in a few cells after 1 hour, a faint evolution of oxygen may commence, and in some cases this is shown at the end or middle of a cell only, over the rest of the cell no evolution of oxygen being shown, but after an hour or so spreading over the whole of the cell. In 2 hours a weak evolution of oxygen is shown from all the living cells, but still no rotation. In 3 hours slow rotation is shown, and after 6 hours all the cells which now remain living show moderately active rotation and assimilation.

The results obtained with *Elodea* and *Utricularia* are given in Table X. It will be noticed that apparently a two days' immersion produces a less marked effect upon assimilation than that produced during the first 16 hours. The reason is that the plants gradually withdraw more and more of the alkali from the solution, which at the same time loses ammonia by evaporation, so that although more than a litre was used in each case, at the end of two days only the very faintest trace of alkalinity was present. In this weakened solution a partial recovery may, if the action has not proceeded too far, take place.

In another experiment the plants were kept for two days in a 0·1-per-cent. solution several times renewed. *Elodea* was entirely killed; in *Utricularia* a few branches remained living and showed no assimilation, after 5 hours in water in a few cases weak but mostly still none, and after 1 day all living branches showed distinct assimilation, in most cases fairly active.



TABLE X.

|                                     | <i>Utricularia vulgaris.</i>                                                                                                                                                                                                                                                                       | <i>Elodea canadensis.</i>                                                                                                                                                                                                                                                                                                   |
|-------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| In 0.1 p. c. solution for 16 hours. | Almost all branches living. No evol <sup>n</sup> of O. In 1 h. mod. active in parts, weak or absent in rest. In 4-6 h. all living branches show distinct evol <sup>n</sup> of O.                                                                                                                   | Most leaves living but no evol <sup>n</sup> of O or rotation. In most leaves apical $\frac{1}{3}$ , though at first living and plasmolysable, dies without recovering. In 1 h. mod. active evol <sup>n</sup> of O at bases of leaves, weak or none in middle portion. In 4 h. distinct assim. is shown by all living cells. |
| In 0.1 p. c. solution for 2 days.   | Plenty of branches entirely or partly living. Most living young branches show weak evol <sup>n</sup> of O, but rest show none. After 5 h. all living branches show weak evol <sup>n</sup> of O, which in some younger branches is active. After 1 day fairly active assim. in all living branches. | In centre of apical tufts leaves with all cells living, and show weak evol <sup>n</sup> of O, which in 2-3 h. is active. Rest of leaves only basal portions living, no evol <sup>n</sup> of O, none after 2 h., weak in 5 h., and in 1 day all living cells show fairly active assim.                                       |

Living plants immersed in dilute acid or alkaline solutions tend in both cases to neutralize them, in the one case removing acid and in the other alkali. When a cell is killed by immersion in a solution of ammonia or ammonium carbonate, it does not contract but is fixed in a condition of rigor, whilst the chlorophyll grains, beyond becoming a somewhat darker green, do not alter, and retain their green colour, provided the solution remains alkaline, for several days without fading. Hence the appearance of the dead cells differs but little from that of a living one, and the loss of plasmolysability is the only reliable indication of death. As we have seen, a cell may remain plasmolysable for a short time after it has totally lost all power of recovery, *i. e.*, a dying cell does not immediately lose its plasmolysability; the complete change from life to death is by no means instantaneous, but involves a series of changes following one another in a certain sequence.



*Effect of Antipyrin upon Assimilation.*

Hauptfleisch\* noticed that in *Elodea* immersion in a 1-per-cent. to 1·5-per-cent. solution of antipyrin causes pre-existent rotation to stop, hence it was to be expected that antipyrin might also have some effect upon assimilation. In a 3 per cent. solution of antipyrin *B. Termo* at once ceases to move, in a 2-per-cent. solution a few Bacteria show slow translatory movement, but in a few minutes all come to rest, whether oxygen is present or not; in 1-per-cent. fewer Bacteria move and not so rapidly as normally; in 0·5-per-cent. the movement is only slightly slowed, in 0·25-per-cent. is hardly at all affected, and in 0·1-per-cent. is as active as in water. Hence observations cannot be made in a strength of solution stronger than 1 per cent.; the recovery is followed after washing and keeping in fresh water.

The results of the experiments made with *Chara fragilis* and *Elodea canadensis* are given in Table Y.

As regards rotation, it must be remembered that in *Chara* the rotation is primary, arising from an internal stimulus which is in continual action, whereas in *Elodea* the rotation is secondary and is only called into action by external stimuli. Leaves of *Elodea*, with the cells in active rotation, if immersed in a 1-per-cent. solution of antipyrin, show in them very slow rotation, and in 2-3 hours none; in 2-per-cent. solution in half an hour a few cells still show slow creeping rotation, and in 1 hour rotation has entirely stopped, but on washing in water it very soon recommences and is active again in a few minutes.

After even 4 hours' immersion in 1-per-cent. or 2-per-cent. antipyrin solution, the chlorophyll grains in *Chara* acquire a distinct brownish-green coloration, and may often, by becoming arranged in lighter (larger interspaces) and darker (smaller interspaces) areas, form an almost regular pattern over the cell. All these cells after 4 hours' immersion remain living and recover, the brownish tinge being still present after 1 day in water, when assimilation is quite active in all cases but disappears in 2-3 days. In *Elodea* all the cells remain living, but neither of these effects are produced. After 8 hours in either 1-per-cent. or 2-per-cent., a few cells in both *Elodea* and *Chara* are killed and do not recover. After 24 hours immersion, in *Elodea* only a

\* P. Hauptfleisch, "Untersuchungen über die Strömung des Protoplasmas in behäuteten Zellen," in Pringsh. Jahrb. 1892, Bd. xxiv. p. 221.



TABLE Y.

|                          | <i>Chara fragilis.</i>                                                                                                                                                                                                           | <i>Elodea canadensis.</i>                                                                                                                                                                                                               |
|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| After 4 hours in 1 p. c. | Rotation present, but slower than normal. Mod. active evol <sup>n</sup> of O. In water in $\frac{1}{4}$ to $\frac{1}{2}$ h. rotation and evol <sup>n</sup> of O are active.                                                      | No rotation. Very weak evol <sup>n</sup> of O, none in parts. In $\frac{1}{4}$ h. in water slow rotation, and in $\frac{1}{2}$ fairly active. Assim. still weak, but in 1 h. both are active.                                           |
| 4 hours in 2 p. c.       | Slow creeping rotation. Evol <sup>n</sup> of O extremely weak, or none. In $\frac{1}{2}$ h. evol <sup>n</sup> of O mod. active, rotation quite active. In 1 h. active assim.                                                     | No assim. or rotation. In $\frac{1}{2}$ h. slow rotation, but no evol <sup>n</sup> of O. In 1 h. rotation active, and evol <sup>n</sup> of O. mod. active, and in 2 h. quite active.                                                    |
| 8 hours in 1 p. c.       | No evol <sup>n</sup> of O, slow creeping rotation. In water in $\frac{1}{2}$ h. weak evol <sup>n</sup> of O and mod. active rotation. Both active in 1 to 2 h.                                                                   | No rotation or evol <sup>n</sup> of O. Rotation active in many cells in $\frac{1}{4}$ h., and faint evol <sup>n</sup> of O, which in $\frac{1}{2}$ h. is weak and in 1 h. active.                                                       |
| 8 hours in 2 p. c.       | No rotation or assim. In $\frac{1}{2}$ h. in water very weak or no evol <sup>n</sup> of O and slow rotation. In 1 h. weak evol <sup>n</sup> of O in all cases, and mod. active rotation. In 2-3 both active in all living cells. | No rotation or evol <sup>n</sup> of O. Rotation active in many cells in $\frac{1}{4}$ h., but still no evol <sup>n</sup> of O. In $\frac{1}{2}$ h. faint evol <sup>n</sup> of O, which in 1 h. is moderately, and in 2 h. quite active. |
| 24 hours in 1 p. c.      | End and cortical cells dead, but over greater part of filaments medullary cells remain living and show in 1 h. weak and in 2 h. fairly active evol <sup>n</sup> of O.                                                            | Almost all leaf-cells living. In $\frac{1}{4}$ h., active rotation in many cells but no evol <sup>n</sup> of O. In $\frac{1}{2}$ h. very weak evol <sup>n</sup> of O in many cases, and in 1 h. weak in all cases, in 2-3 h. active.    |
| 24 hours in 2 p. c.      | Plenty of medullary cells living and showing in 1 h. faint, in 2 h. weak, and in 3-4 h. active evol <sup>n</sup> of O.                                                                                                           | Few leaf-cells at base and mid-rib living and showing in $\frac{1}{4}$ h. active rotation. After 1 h. no evol <sup>n</sup> of O, weak in 2 h., and in 4 h. mod. active in parts. In 1 day all living cells show distinct assim.         |

few cells remain living, and in *Chara* only the medullary cells. After the latter have been kept for 2 or 3 days in water exposed to light the cortical cells bleach and become transparent, when the green rotating and assimilating medullary cells come distinctly into view and may remain living and assimilating for more than a week after the rest of the plant has been killed.



In the preceding paragraphs we have seen that the function of assimilation may be temporarily or permanently inhibited in a variety of ways—by cold, by heat, by desiccation, by asphyxiation, by etherization, and by treatment with acids, alkalies, or anti-pyrin. Boussingault was the first to notice that asphyxiation causes a stoppage of assimilation, but erred in supposing that the stoppage was always permanent and necessarily involved a loss of vitality. Boussingault's "asphyxie" is really only the last stage of asphyxiation.

Pringsheim has shown by experiments with *Chara* that it is possible to produce a condition of partial asphyxiation or inanition during which both assimilation and rotation cease. He concludes that inanition is produced by the absence of oxygen and that recovery can only take place if a supply of oxygen is introduced before inanition has passed into asphyxia, but does not take place if the specimen is exposed to light in an atmosphere of hydrogen containing a trace of  $\text{CO}_2$ . Working from this latter assumption, Pringsheim comes to the conclusion that assimilation is a purely protoplasmic function, being hence intimately bound up with rotation, ceasing when it ceases and recommencing when it recommences, and that the oxygen derived from the assimilation of  $\text{CO}_2$  exists in the cell in the form of an oxygen-producing substance which undergoes decomposition and gives off oxygen on the outer surface of the cell only, no free oxygen being present in the interior of the cell\*.

In support of his assumption that an oxygen-producing substance is present in every assimilating cell, Pringsheim evidences the fact that dead green and non-green cells commonly at death show an evolution of oxygen which can be detected by the Bacterium method. This statement evidently required further proof, as the movement of *B. Termo* might be affected by the nutritious substances which exude from the dying and contracting cell-contents. As a matter of fact, it was found that the nutritious substances evolved from a dying cell by exosmosis do aid the movement of the Bacteria and enable them to continue moving in a closed cell longer than they would have done in water alone, but that a distinct evolution of oxygen may take place from a dying cell previously actively assimilating.

In a closed cell in water *B. Termo* ceases to move in 2 or

\* That free oxygen is actually present in the interior of the cell has now been definitely proved. See Pfeffer, "Oxydations Vorgänge in lebenden Zellen," in *Abh. Sachs. Ges. Wiss. Leipzig* (1889), pp. 375-516.



3 minutes; in neutral meat-extract or in a boiled watery extract of moss-leaves it takes 15 to 20 minutes longer to come to rest; whilst in the neighbourhood of dying cells which have been suddenly fatally injured the movement may continue, provided the cell was previously actively assimilating, for as long as 2 hours, and is independent of light. Cells killed by asphyxiation and non-chlorophyllaceous cells show no evolution of oxygen on death. The possibility of a slight evolution of oxygen taking place in many cases coincidently with death does not necessarily involve the assumption of the presence of a special oxygen-producing substance in the living assimilating cell. The slight amount of oxygen set free may have been held in a state of loose combination by the protoplasm, or the phenomenon may be a purely physical one, the actively assimilating cell when suddenly killed continuing for some time to slowly diffuse out the oxygen with which its substance was previously saturated.

Pringsheim's statement that recovery from inanition will not take place if the plants are exposed to light in an assimilatory mixture of hydrogen and  $\text{CO}_2$ , but only when free oxygen is admitted, is not true in the case of Mosses. The phenomena he observed in support of this contention, with *Chara*, can readily be explained when we remember that the inhibition of assimilation caused when inanition is produced will necessarily be followed by a longer or shorter latent period, and if we suppose that the addition of free external oxygen exercises a stimulating influence and shortens the latent period of recovery. The conditions as regards the absorption and evolution of oxygen are very different in an atmosphere of hydrogen containing a trace of  $\text{CO}_2$  (such as Pringsheim experimented with) to what they are in ordinary air. In such an atmosphere, the oxygen's partial presence being *nil*, the cell will rapidly evolve all dissolved oxygen it may contain, an abnormal condition being thereby produced. This is shown by the fact that in preparations of *Chara*, even though exposed to continuous illumination, in a current of Hydrogen + a trace of  $\text{CO}_2$ , which continually removes from them all oxygen as fast as it is formed, inanition is finally produced and assimilation and rotation cease. Moss-leaves, however, under such conditions retain the power of assimilation for an indefinite length of time.

Engelmann \* explains the phenomena on which Pringsheim's

\* "Die Purpurbakterien und ihre Beziehungen zum Licht," in Bot. Zeit., Oct. 1888, p. 717.



reasoning is based, by supposing that the chlorophyll grains, normally accustomed to an abundant oxygen supply, are readily affected and soon killed by the deficient supply of oxygen, whilst the protoplasm, being more resistant, remains living and streaming. This, though partly true, is only a partial explanation. The condition first produced in the chlorophyll grain is one of inanition, characterized by an inability to assimilate, but from which recovery is possible. If the exposure goes too far, the chlorophyll grains are killed and lose their power of recovery, and this is quickly followed by the death of the entire cell.

That even in *Chara* a supply of external oxygen is not always necessary for the recovery from inanition is shown by the fact that end cells of *Chara* which had temporarily lost the power of assimilation, if kept in carefully closed cells under water exposed to light, recovered and recommenced to assimilate.

If preparations of *Chara* are kept in closed cells thinly ringed with vaseline in the darkness, in many cases the end cells remain living and show distinct though slow rotation for many days, and in certain cases for as long as five weeks, though exposed to light for less than one minute each week. No loss of water takes place from such a cell; *Bacterium Termo*, if enclosed, comes to rest in a few minutes, and as the ringing remains intact it is difficult at first to explain the phenomena observed, for here apparently the rotation of *Chara* continues in the absence of oxygen for as long as several weeks, which is in direct contradiction to the results obtained in an atmosphere of hydrogen. It does not follow that because *Chara* in an atmosphere of hydrogen ceases to show rotation and finally dies, that therefore this effect is produced by the absence of oxygen, for it might readily be due to a direct action of the hydrogen itself. If, however, the preparations are carefully and thickly ringed with a mixture of vaseline and wax, or vaseline and paraffin, or if a considerable quantity of living *Bacterium Termo* is enclosed, in two or three days the end cells cease to show rotation and die. *Spirilla* and *Monas Termo*, for the movement of which the presence of the minutest trace of oxygen suffices, continue to move in thinly ringed preparations as above for an indefinite length of time, whereas in thickly ringed cells they cease to move in a few hours. Through the vaseline in the thinly ringed preparations, therefore, a trace of oxygen diffuses, and this is



sufficient to cause the movement of *Spirillum* and the rotation of *Chara* to continue, but is insufficient for the movement of *Bacterium Termo*.

Preparations of *Chara*, thickly ringed with a mixture of wax and vaseline and kept immersed in cold boiled oxygenless water in closed bottles in the darkness, gave the following results:—

|                    |                                                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| After<br>20 hours. | All end cells living. In half no rotation, in rest slow creeping rotation. After a few minutes exposure to light, rotation quickens in those cells where it was previously present, and a weak evolution of oxygen is shown. In some cases a weak but distinct evolution of oxygen is shown before rotation commences. After 15 to 30 minutes' exposure to light, both rotation and assimilation are fairly active. |
| After<br>30 hours. | Most end cells are still living, but no rotation or assimilation is shown. Rotation soon returns on exposure to light, but assimilation mostly not for an hour or two. In some cases no recovery at all takes place. In a few cases slow rotation is shown after exposure to light, but assimilation does not recover and, later, rotation ceases and the cells die.                                                |
| After<br>40 hours. | All cells are fatally affected and have lost the power of recovery.                                                                                                                                                                                                                                                                                                                                                 |

In an atmosphere of hydrogen in the darkness all rotation ceases in *Chara* in about 8 or 10 hours, and in a current of hydrogen exposed to light is in from 10 to 14 hours extremely slow or absent. In a current of hydrogen, oxygen is continually being forcibly withdrawn from the filaments, as is shown by the fact that, when suddenly surrounded by an atmosphere of hydrogen, the filaments give off oxygen more rapidly than before, causing an increased activity of the movement of the surrounding Bacteria, and continue to do so for some time, especially if exposed to light. In the closed cell surrounded by oxygenless water, on the other hand, rotation stops, solely because all supply of oxygen from without is cut off, and ceases only when all stored intramolecular oxygen has been used up and the protoplasm has been reduced to its lowest possible condition of deoxidization.

If preparations of end cells of *Chara* are exposed to light in a hanging drop with *B. Termo* in a gas-chamber filled with pure CO<sub>2</sub>, in about 10 minutes all evolution of oxygen\* ceases, but

\* *B. Termo*, in a hanging drop over an atmosphere of CO<sub>2</sub> containing a trace of oxygen, continue to move fairly actively, though after prolonged exposure the movement is weakened and finally stops.



very slow rotation still persists. In 20–30 minutes rotation also ceases. If now a rapid current of hydrogen is passed through till only mere traces of  $\text{CO}_2$  are left, in 5 minutes a distinct evolution of oxygen is shown and slow rotation has commenced. In 15 minutes the evolution of oxygen is quite active, and in half an hour rotation is also quite active. In such experiments no external supply of oxygen reaches the filaments, and if Pringsheim's contention that free oxygen is never present in the interior of a cell were true, then this would be a case in which a recovery of both assimilation and rotation takes place without any oxygen being supplied from without, the oxygen necessary for the performance of rotation as well as of the other protoplasmic functions being supplied from the first by the assimilation of the cell itself. The experiment is, however, faulty, because the stoppage of the protoplasmic activity is due to a direct poisonous influence of the  $\text{CO}_2$ , and certainly takes place before the amount of free or loosely held oxygen present in the cell is reduced to its lowest ebb. It is quite possible that, if this latter condition were actually produced, no return or recommencement of rotation or assimilation (taking for granted that in some of its stages at least assimilation is a plasmatic function) could occur without being started by a supply of oxygen from without. Such a condition, in which all free or loosely combined oxygen in the cell is removed down to the last molecule, seems to be rather a theoretical than a practical one, being impossible to produce without at the same time destroying the protoplasmic vitality.

There can be little doubt that assimilation is largely if not mainly a protoplasmic function, and that the chlorophyll pigment is simply a cooperative member in the assimilatory apparatus of the living chlorophyll body. Whether the function is located entirely in the plasmatic stroma of the chlorophyll grains, or also partly in the protoplasm immediately surrounding these, or in the general protoplasm of the cell, is immaterial to the question at issue, though all the evidence goes to show that assimilation in the special sense is restricted entirely to the chlorophyll grain. Of all the different modes of inhibiting assimilation described in the previous pages, two only are accompanied by a change in the colour of the chlorophyll, viz. the winter browning of evergreens and the effect of antipyrin on



*Chara*. Of these, the latter is only very slight and does not, as a matter of fact, perceptibly affect the assimilatory powers of the cell. In the former case the inability to assimilate is, where the browning and decolorization is complete, readily explained by supposing that the absorption of the necessary energy cannot take place, assimilation returning as soon as the necessary pigment is formed and the necessary connection established, which is often whilst the plant is still quite yellow. What is certain is that the chlorophyll bodies, being living plasma organs, are, like all living plasma, liable by various disturbing influences to an arrest of certain functions, in this case more especially of assimilation. Such functional derangement is possible with and without visible alteration in either the colour or structure of the chlorophyll grain. In this respect the absorption or non-absorption of light is of only secondary importance, for chlorophyll grains of normal appearance and showing normal absorption may be temporarily inactive.

The question as to whether an isolated chlorophyll body can assimilate and evolve oxygen is, in this connection, of much interest. Engelmann\* states that single isolated chlorophyll bodies continue, if exposed to light, to assimilate and evolve oxygen, but gives no further details beyond stating that it is shown by the chlorophyll grains of many plant-cells, and that in water the chlorophyll grain swells and bursts and the evolution of oxygen ceases. In one case given, viz. *Hydra viridis*, we have to do not with chlorophyll grains but with Zoochloanthellæ, modified endophytic algal cells, according to Brandt†. Haberlandt‡, however, found that in *Funaria hygrometrica* isolated chlorophyll grains continue to assimilate and evolve oxygen (Bacterium method) in a 25-per-cent. solution of cane-sugar, but does not state how long the power of assimilation is retained. His experiments are also open to the serious objection that in a 25-per-cent. solution of sugar *B. Termo* is plasmolysed and almost entirely ceases to move. The explanation probably is that, in making the preparations, the sugar solution was diluted

\* Engelmann, "Neue Methode zur Untersuchung der Sauerstoffanscheidung pflanzlicher und thierischer Organismen," in Bot. Zeit. 1881, p. 446.

† Brandt, in Monatsber. d. Berlin. Akad. 1881, p. 388.

‡ Haberlandt, 'Ueber die Beziehungen zwischen Function und Lage des Zellkernes,' 1887, p. 117.



down to 15 per cent. or 20 per cent., in which *B. Termo* continues to move actively.

The method adopted is to cut sections with a sharp razor in sugar solution, when, on removing the sections, many uninjured chlorophyll grains are left behind. To this fluid Bacteria, taken fresh from a pure culture on the point of a sterilized platinum wire, are added, and the cell is then closed. The less manipulation the better, no pressure from the cover-glass is allowable, air-bubbles must be totally absent, the Bacteria must be from young and perfectly pure cultures, the sugar solution filtered and sterilized, and the leaves carefully washed and brushed in sterilized water. Otherwise, preparations from moss-leaves often contain unicellular algæ, which naturally show active assimilation, and which are in some cases rather like chlorophyll grains and apt at first to be mistaken for them, being surrounded by a cell-wall of extreme tenuity. When we consider to what severe treatment the chlorophyll grains must necessarily be subjected in order to procure its isolation, and under what abnormal conditions it then is, it is not surprising to find that the majority of the plants experimented with, including *Chara*, *Nitella*, *Oxalis*, *Ilex*, &c., gave negative results. In *Oxalis* the acidity of the liberated cell-sap probably injuriously affects the chlorophyll grain, and this may to a greater or less extent often be the case. The cases in which definite results were obtained are arranged for convenience in tabular form :—

|                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Catharinea undulata.</i>  | Weak assimilation in 10 p. c. sugar, stronger in 15 p. c. In 1 to 3 hours the evolution of oxygen begins to weaken and in 3 to 5 hours ceases. In 1 p. c., 2 p. c., 3 p. c., 4 p. c., or 5 p. c. $\text{KNO}_3$ solution no power of assimilation is shown.                                                                                                                                                                                                                                   |
| <i>Funaria hygrometrica.</i> | In 10 p. c. and 15 p. c. many chlorophyll grains show weak but distinct assimilation, which in 1-2 hours weakens and in 3-5 hours ceases. Similar results with the chlorophyll grains of the sporogonium, but being smaller the evolution of O is correspondingly weaker. [Any spores present show a distinct evolution of oxygen when in the young green chlorophyll containing condition, but this becomes weaker as the spores grow older, and the chlorophyll alters and finally ceases.] |
| <i>Dicranum scoparium.</i>   | In 5 p. c. and 10 p. c. cane-sugar very weak assimilation is shown in many chlorophyll grains, but in 1 hour this begins to weaken, and in 2-3 hours none is perceptible.                                                                                                                                                                                                                                                                                                                     |



Table (*continued*).

|                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Vallisneria spiralis.</i>  | In Pfeffer's Nahrlosung (15 times concentrated) weak but distinct evolution of O from many chlorophyll grains in light, ceasing in darkness, but power of assimilation is soon lost. In 4 to 5 p. c. cane-sugar evolution of O almost imperceptible and soon ceases. In 10 p. c. cane-sugar, distinct but weak assimilation in many cases, which after 3 hours begins to weaken, and in 4 to 6 hours has entirely ceased.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <i>Selaginella helvetica.</i> | Both large and small chlorophyll grains may show evolution of O, former when uninjured better than the latter, owing to their larger size. At best never more than $\frac{1}{2}$ to $\frac{1}{3}$ the activity of the evolution of O from an algal cell of the same size. In 15 p. c. sugar solution in many cases assimilation still quite distinct after 5 or 6 hours, and in few cases still present, though much weaker after 10 hours, but the next morning the grains are always dead. In 10 p. c. sugar, the evolution of O is less marked, in 3 hours is very weak, and is shown in only few cases, in 5 hours ceases.<br>In 2 p. c. and 3 p. c. $\text{KNO}_3$ weak evolution of O, which ceases in 2-3 hours, and is present in only relatively very few quite uninjured grains.<br>In 5 p. c. $\text{K}_2\text{C}_4\text{H}_4\text{O}_6$ , weak evolution of O from most uninjured grains, but in 1-2 hours ceases. |

The evolution of oxygen very rarely approaches in strength to that shown by an algal cell of similar size and is nearly always much weaker. When the evolution of oxygen is relatively fairly active and the chlorophyll grain is lying free in the fluid, the Bacteria swarming around it may cause it to roll about or twist on one axis from time to time. When it is very weak, a Bacterium lying on or near the chlorophyll grain may be seen to suddenly dart off for a short distance; if the path is short it may return, but if long it may lose itself in the surrounding oxygenless desert and come to rest. This is repeated at intervals by other neighbouring Bacteria.

In darkness the movement entirely stops in a minute or two, and on re-exposure to light is shown again almost at once, recommencing in less time than it takes to cease. The stoppage and recommencement may be repeated many times with the same chlorophyll grain. Young and old, larger and smaller, dividing and non-dividing chlorophyll grains may show the same evolution of oxygen, but naturally it is more marked where the chlorophyll grain is larger. The fact that the evolution of oxygen ceases in the dark and recommences on exposure to light, shows that it is not due to the setting free of loosely combined



oxygen previously present in the chlorophyll grain, nor is the movement of the Bacteria due to the evolution of nutritious substances from the chlorophyll grain, for in a solution of sugar the Bacteria come to rest as soon in the neighbourhood of dying cells or grains as in the open field.

To detect the evolution of oxygen, chlorophyll grains quite free from adhering protoplasm are better than ones to which plasma is attached. The dying but still respiring plasma absorbs oxygen, and this is the case even with the free entire (but fatally affected) cell-contents; such show in sugar solution no evolution of oxygen and no movement of Bacteria in their neighbourhood (previously to examination the plants were in all cases kept for one to a few days in darkness, or semi-darkness). Rarely the complete uninjured cell-contents may be obtained lying free. These show a distinct power of assimilation which may continue for several hours, and in a few cases for more than a day.

In the cell with Bacteria the chlorophyll grains may die because of the abnormal conditions (absence of oxygen, presence of Bacteria) to which they are exposed in the closed cell. Preparations of chlorophyll grains, however, kept in a hanging drop of sugar solution in a dark chamber for several hours are found to show no evolution of oxygen on examination; *i. e.* the chlorophyll grains are either unable to recover from the shock of removal and isolation, or are incapable of continuing a separate existence apart from the cell to which they belong for longer than a few hours, their autonomy being only partial and incomplete.

In other media than sugar solution similar or worse results are given. Sugar solution + 1 per cent. peptone or with a little gum arabic gives similar results to sugar solution alone, whilst the viscosity of egg-albumin stops or so retards the movement of the Bacteria as to prevent its being used as a medium.

In *Selaginella*, owing to the plants having been previously kept in the darkness for 2 or 3 days, the chlorophyll grains show no perceptible starch. The isolated chlorophyll grains, after showing 10 hours' assimilation, on staining with iodine show very minute granules of starch, but not always. These might, however, have been originally present, but invisible until stained, or might be formed from the sugar solution in which the grain lies. In preparations kept in darkness for the same time, however, no formation of starch can be detected. In *Vallisneria* a similar



formation of starch can apparently be detected if, as rarely happens, the chlorophyll grains assimilate for longer than 4 hours, but the grains are excessively minute and still more doubtfully the result of the assimilation of the chlorophyll grains after isolation.

In *Elodea canadensis* the externally isolated chlorophyll grains show no power of assimilation. If leaf strips are plasmolysed in strong sugar solution and then examined in 15 per cent. to 20 per cent. sugar solution + Bacteria, the shrunken cell-contents do not perceptibly re-expand, but show a distinct though not strong evolution of oxygen. In some cells, at one end or corner 2 or 3 chlorophyll bodies or even single ones may be isolated, and from these a weak but distinct evolution of oxygen can be detected if the plasmolysed bodies of the neighbouring cells are sufficiently distant, and if the chlorophyll grains are adhering to the upper side of the cell-wall. In some cases such isolated chlorophyll grain or grains can be seen to have a little plasma associated with them, or to have plasmatic connections, but in other cases appear to be perfectly free from plasma and to be without any fibrillar connection with the rest of the cell contents, and nevertheless retain the power of assimilating and evolving oxygen.

It appears, therefore, that in certain cases an isolated chlorophyll grain can continue to assimilate, but is incapable of an independent life, ceasing to assimilate, and dying within a few hours after removal from the parent cell.

The above fact does not in any way support the hypothesis that the chlorophyll bodies of higher green plants are phylogenetically to be regarded as endophytic symbiotic algal cells which have completely or almost completely lost their autonomy, but is rather comparable with similar cases known in animal physiology (Frog's heart or sartorius or gastrocnemius muscle), where an isolated organ of considerable physiological complexity may remain living and functionally active for many hours, or even a couple of days, after removal from the parent body.

Cases in which in adult cells, after a destruction of the "primary" chlorophyll grains, a new formation of "secondary" chlorophyll grains takes place are not definitely established. Thus Schimper has shown that, in the winter browning of evergreens, it is only when the chlorophyll bodies remain intact that



the cells can recover and become green again. Similarly the secondary chlorophyll grains which Dehnecke (*l. c.*) describes as appearing in many cases after the primary chlorophyll grains, loaded with starch, have lost their starch, disintegrated and disappeared, are either formed from the latter or are simply the same grains in a different condition. On the other hand, the fact that in young cells and organs chlorophyll corpuscles may be formed and differentiated where none were previously present (Schimper)\*, is proof positive that the chlorophyll corpuscle is simply a specially modified and differentiated portion of the protoplasm of the cell, resembling the nucleus somewhat in the structure and nature of its plasma and in being bounded (according to some authors) by a distinct limiting membrane, but differing in its infiltration with chlorophyll and in being specially differentiated to form the centre or focus for the process of assimilation.

Though the chlorophyll body is specialized simply and solely for assimilation, it does not follow that this function will not be affected by changes in, or by causes influencing, the general protoplasm of the cell. It is only the first stages of plant assimilation which are restricted to the chlorophyll body, those which consist in the conversion of simple inorganic compounds (water and  $\text{CO}_2$ ) into more complex and organic compounds (sugar and starch); the final processes of assimilation, in which dead organic or inorganic compounds are built up into living protoplasm, are carried on by the general protoplasm of every cell, whether animal or plant. Any cause which acts upon the general cell protoplasm and stops or hinders either the later stages of assimilation or the removal of the accumulated assimilatory products, will also react upon the first stages of assimilation, *i. e.* assimilation by the chlorophyll body, hindering or stopping this also. Thus Saposchnikoff\* has shown by experiments with cut leaves of *Vitis* and *Rubus*, that when the carbohydrates present reach a certain percentage no more are produced. In *Vitis Labrusca*

\* A. F. W. Schimper, "Untersuchungen über die Chlorophyllkörper," in Pringsh. Jahrb. Bd. xvi. 1885.

† W. Saposchnikoff, "Ueber die Grenzen der Anhäufung der Kohlenhydrate in den Blättern der Weinrebe und anderen Pflanzen," in Ber. d. D. Bot. Ges. Bd. ix. 1891, p. 293. Also preceding papers in 1890 and 1889, p. 258 and p. 233.



this takes place when the amount of carbohydrate (starch and sugar) reaches from 17 per cent. to 25 per cent. the dry weight of the leaf. As soon as a leaf-cell contains 2 per cent. of sugar the conversion of this into starch commences, but when the amount of starch has reached its maximum, the leaf-cell still continues to assimilate until it contains sugar of from 6-per-cent. to 8-per-cent. concentration, and is no longer plasmolysable by a 10-per-cent. solution of glycose.

In a later paper Saposchnikoff\* finds that isolated leaves of *Vitis vinifera*, after 6 to 7 days' exposure to light, can form no more carbohydrates, and then contain of carbohydrates 23 per cent. to 29 per cent. of the dry weight of the leaf, there being very little difference between the results given by leaves with their stalks in distilled water and others with their stalks in a nutrient solution. If the leaves are kept in an atmosphere containing at the commencement 20 per cent of CO<sub>2</sub>, in 3 days the maximal accumulation of carbohydrates is produced, and this is from 30 per cent. to 35 per cent. of the dry substance of the leaf, this greater amount being probably due to the leaf remaining more normal in the shorter time of exposure.

If the accumulation of carbohydrates eventually causes a stoppage of assimilation, it must also involve a cessation of all evolution of oxygen. Hence the Bacterium method affords a ready means of confirming and extending Saposchnikoff's observations, especially as regards the recovery or non-recovery of assimilation on the diminution or removal of the accumulated carbohydrates.

*Effect of the Accumulation of the Assimilatory products  
(Carbohydrates) upon Assimilation.*

The adult leaves cut with the upper part of the petiole attached are kept with their stalks in water exposed to the brightest diffuse light, but shielded from too intense and prolonged sunlight.

\* "Beitrag zur Kenntniss der Grenzen der Anhäufung vom Kohlenhydraten in den Blättern," in Ber. d. D. Bot. Ges. Bd. xi. 1893, p. 391.



|                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Rubus odoratus.</i>                              | After 10 days the leaves show distinct but weak evolution of O, and contain abundance of starch. If then kept for 2-3 days in darkness at 30° C., a distinct diminution of the starch and a moderately active evolution of O are shown. After 15 days the leaves are yellowish in parts, but even the normal green parts show no evolution of O, and the starch is very abundant. If kept in darkness for 3 days at 30° C., all the leaves which remain normal and living show a fairly active evolution of O and a marked diminution of the amount of starch present. |
| <i>Vitis vinifera</i><br>and <i>Vitis Labrusca.</i> | After 10 days a weak evolution of O is still shown, but in parts none. After 12 days no evolution of O, the cells are loaded with starch, but the chlorophyll grains are otherwise normal. After 3 days in darkness at 30° C., a fairly active evolution of O is shown in all cases and the starch is distinctly diminished in amount.                                                                                                                                                                                                                                 |
| <i>Æsculus Hippocastaneum.</i>                      | After 7 days evolution of O is quite weak. After 10 days the mesophyll at bases of leaflets in parts turns yellow, rest of leaf green and normal, but no evolution of O. In darkness for 3 days at 30° C., the greater part of the leaf remains green and shows a moderately active evolution of O, the starch being distinctly reduced in quantity.                                                                                                                                                                                                                   |

It is not always possible to secure a return of assimilation by keeping the plants in darkness and allowing the respiratory activity of the leaf-tissues to use up a certain amount of the accumulated carbohydrate, and permit a resumption of assimilation, for the leaf may have been so injuriously affected by the treatment to which it has been subjected, that it may die when kept in darkness without any appreciable amount of carbohydrate being removed or any return of assimilation taking place.

In an atmosphere containing a large, but not too large, percentage of CO<sub>2</sub>, since assimilation goes on more actively, it is natural to expect that in the isolated leaf the maximal accumulation of carbohydrates and consequent stoppage of assimilation will take place with much greater rapidity than in ordinary air. Thus adult leaves of *Vitis vinifera* and *V. Labrusca* were exposed as previously, but in a bell-jar in an atmosphere containing 10 per cent. of CO<sub>2</sub>, renewed daily, the plants being shielded from too intense sunlight, and the temperature within the bell-jar never being allowed to rise above 30° C. At the end of 3 days the leaves still show in parts a weak evolution of oxygen, but in parts none. After 4 days the leaves are quite green and normal, but the cells are loaded with starch and no evolution of



oxygen is shown. After being kept in darkness for 3 days at 30° C., the leaves are still green and normal in appearance, there is a marked diminution in the amount of starch present, and a fairly active evolution of oxygen is shown when exposed to light. That the more rapid stoppage of assimilation is due simply to the more rapid accumulation of carbohydrates, and not to any poisonous depressant influence of the CO<sub>2</sub>, is shown by the fact that leaves of *Vitis vinifera* and *V. Labrusca* kept in an atmosphere containing 10 per cent. of CO<sub>2</sub>, but exposed to very weak diffuse daylight, show after 4 days no perceptible accumulation of starch and an active evolution of oxygen on examination.

In experimenting with Mosses, it is practically impossible, if the entire plants are used and kept in ordinary air, by even full and prolonged exposure to light to cause a maximal accumulation of carbohydrates and consequent stoppage of assimilation, for the starch and sugar are being continually transferred to the growing parts and used there for the formation of proteid and cellulose. If, however, the young growing apices are removed and plants kept stuck through thin slips of cork floating on water, isolated from one another, fully exposed to light, and in an atmosphere containing from 10 per cent. to 15 per cent. of CO<sub>2</sub>, quite definite results are obtained. Thus after 5-6 days exposure the leaves all remain living, but show no evolution of oxygen (*Dicranum scoparium*, *Catharinea undulata*, *Bryum caespitium*, *Mnium stellare*). In *Catharinea* the cells are quite granular, with starch grains both enclosed in the chlorophyll grains and lying free in the protoplasm. In *Bryum* the chlorophyll grains are swollen with one or more centrally placed starch grains, and abut on one another so as to be polyhedral instead of spherical as normally. In *Mnium* the protoplasm is finely granular, but the granules do not stain with iodine. Starch is present only in the chlorophyll corpuscle and in the form of a single large central grain. In *Dicranum* the protoplasm is granular, opaque, and contains an abundance of oil, whilst each chlorophyll grain contains a rather small but distinct starch grain, commonly absent from normal plants. Here starch appears only to be formed when an excess of oil and sugar are present. After the plants have been kept for 1 day in darkness at 30° C. in a damp well-aerated atmosphere, a weak power of assimilation is shown by most leaves of *Mnium* and *Bryum*, at the bases of the leaves in *Catharinea*, and in the younger leaves



of *Dicranum*, though no perceptible diminution of the stored starch has taken place. After 3 days a fairly active power of assimilation is shown by all living leaves, and a perceptible diminution in the amount of starch present has taken place. If the same experiment be repeated with entire plants, after 8 days nearly all adult leaves can no longer assimilate, but the young apical leaves still show a weak to fairly active power of assimilation.

Experiments with *Allium Cepa* are interesting, because in this plant the superfluous carbohydrate is stored not in the form of starch but of sugar. The leaves of *Allium* die rather readily when separated, and hence form unfavourable experimental material, but if the fully-developed flowering axis be cut off at its base, and the flower-head removed, the axis may remain living for weeks if the basal end be kept in water. Fehling's test shows that in such a fresh flowering-axis traces of sugar are present in the outer assimilating layers, but much more in the inner parenchyma and abundance in the basal  $\frac{1}{6}$ th to  $\frac{1}{10}$ th of the axis. After being exposed to bright light for 8 days (base in weak sugar solution) or 14 days (base in water), sugar is present in all cells and tissues and only a weak power of assimilation is shown. After 10 days (base in sugar) or 14 days (base in water) all the cells are rich in sugar, and no evolution of oxygen is shown, though the cells are normal in appearance and the chlorophyll grains quite green and unaffected. After 2 days in darkness at 30° C. a very weak power of assimilation is shown, and plenty of sugar is still present in the inner parenchyma, but much less in the outer green assimilating layers. After 4 days assimilation is moderately active, and a little sugar is still present in the outer assimilating layers, but more in the inner parenchyma.

Plants of *Ædogonium* kept in water through which CO<sub>2</sub> is occasionally allowed to slowly bubble, and exposed for several days to bright diffuse daylight, become packed with starch and show no power of assimilating except in a few cells, chiefly at the growing ends of the filaments, but also in intercalary cells, which contain much less starch, and show a moderately active evolution of oxygen. If the plants are now kept in darkness in well-aerated water at 20° C., after 5 days many filaments die, but most remain living, show a distinct reduction in the amount of starch present, and a fairly strong power of assimilation.



With most water-plants it is difficult to cause the maximal accumulation of carbohydrates to take place, but if plants of *Elodea canadensis* and *Utricularia vulgaris* are kept exposed to bright light in a frequently renewed 5-per-cent. solution of grape-sugar, in many of the older leaves or branchlets a marked accumulation of starch takes place and the power of assimilation is correspondingly weakened, in some cases none being perceptible. If moss-plants (*Dicranum scoparium*, *Bryum caespititium*) are kept partly immersed in a 5-per-cent. solution of grape-sugar, and exposed to bright light, a maximal accumulation of carbohydrates and a consequent cessation of assimilation until the amount is reduced, is readily produced in the older adult leaves.

Since Saposchnikoff has shown that in isolated leaves of *Vitis*, when the further formation of carbohydrates ceases, the amount of sugar present in the cell-sap may reach in strength that of a 10-per-cent. solution, it might be expected that mere immersion in such a solution of sugar would cause a stoppage of assimilation. Klebs \* has, however, shown that various Algæ and even Mosses (*Funaria*) cultivated in 20-per-cent. cane-sugar or 10-per-cent. glucose may, though plasmolysed, form a new cell-wall and starch grains, but his experiments are not conclusive as to whether the plants actually assimilated or merely converted absorbed sugar into starch. To settle this point, direct observations by the Bacterium method were necessary. As regards the movement of the test Bacteria in strong solutions of sugar, &c., Fischer † has shown that *Spirillum* shows in 1-per-cent.  $\text{KNO}_3$  or 5-per-cent. cane-sugar weak plasmolysis in a few cases, in 2-per-cent.  $\text{KNO}_3$  or 10-per-cent. cane-sugar strong plasmolysis in nearly all cases, and very strong plasmolysis in 5-per-cent.  $\text{KNO}_3$  or 30-per-cent. cane-sugar, whilst in water the Bacterial protoplasm re-expands and the Bacteria become homogeneous again. The movement of the Bacteria ceases only when they are strongly plasmolysed. Beyond stating that *Bacterium Termo* (*Bactrillum Pseudotermo*) is strongly plasmolysed by a  $2\frac{1}{2}$ -per-cent. solution of  $\text{KNO}_3$ , and that in almost any nourishing solution it retains the power of movement, even in a very weak acid culture in which other Bacteria lose the power of movement, no

\* G. Klebs, "Beiträge zur Physiologie der Pflanzenzellen," in Tübingen, Bot. Untersuch. Bd. ii. Hft. 3, 1888, pp. 500-537.

† Alfred Fischer, "Untersuchungen über Bakterien," in Pringsh. Jahrb. Bd. xxvii. 1895, pp. 9, 31.



further information is given concerning the persistence of the power of movement in this form.

Actual observation shows that the movement of *Bacterium Termo* remains quite active in 1-per-cent.  $\text{KNO}_3$ , 10-per-cent. cane-sugar, or 5-per-cent. grape-sugar; in  $2\frac{1}{2}$ -per-cent.  $\text{KNO}_3$ , 20-per-cent. cane-sugar, and 10-per-cent. grape-sugar, fewer Bacteria move and the movement is slower; in 3-per-cent.  $\text{KNO}_3$ , 25-per-cent. cane and 15-per-cent. grape sugar, a few Bacteria still show distinct but slow translatory movement, but only a very slight attraction to air-bubbles; in 5-per-cent.  $\text{KNO}_3$ , 30-per-cent. cane-sugar and 20-per-cent. grape-sugar, all translatory movement ceases. If the Bacteria have been kept in ordinary sterilized tap-water for 2-3 hours, they show almost undiminished activity of movement, but now in a 20-per-cent. solution of cane-sugar, instead of a 30-per-cent. solution as with Bacteria fresh from Agar-tube cultures, all movement at once ceases. If sterilized solutions of cane and grape sugar, 10-per-cent., 20-per-cent., 30-per-cent., and 40-per-cent., are inoculated with *B. Termo*, either the cultures do not develop, or, if they do, the Bacteria are all immotile forms and show no movement even when the solutions are diluted down to 5 per cent. Hence, though the ability of the Bacterium to retain its power of movement in strong solutions of sugar is weakened when kept for a few hours in water, the converse does not hold, for cultures made in strong sugar solutions show no power of movement at all.

It is possible, however, to conduct accurate direct observations by the Bacterium method upon the absence or persistence of assimilation in solutions of 10-per-cent. to 15-per-cent. grape-sugar or in 20-per-cent. grape-sugar.

Experiments with *Elodea*, *Utricularia*, *Bryum*, *Funaria*, and *Allium* show that in solutions of these strengths the power of assimilation remains fairly active as long as the cells are living, though in *Elodea* the cells may be completely plasmolysed, the plasmolysis being less marked in *Bryum* and *Funaria*, still less in *Allium*, and not at all in *Utricularia*. Assimilation is naturally not so active in a 20-per-cent. solution of cane-sugar as it is normally, but the weakening is probably simply due to physical causes, viz. the withdrawal of water and the lessened exposure of the chlorophyll grains in the plasmolysed condition. It does not follow that because cells are immersed in a strong sugar solution that therefore the cell-sap will contain a similar percentage of sugar and thereby cause, if sufficiently concentrated, a stoppage



of assimilation. The primordial utricle in all cases determines at what rate and in what quantity any given substance will pass through to the cell-sap. Immersion in a weak sugar solution helps to produce the maximal accumulation of carbohydrates more rapidly only because it affords another source from which carbohydrate material is drawn, and not because of any physical diffusion through the primordial utricle and accumulation in the cell-sap.

Immersion in very strong sugar solution causes so marked a withdrawal of water as to affect the power of assimilation, and finally the vitality of the cell. The plants are placed in solutions of gradually increasing strength until the desired percentage is reached, when, after a few hours' immersion, preparations are examined in a 20-per-cent. solution of cane-sugar with Bacteria. After being for half an hour in 20-per-cent. and the same time in 10-per-cent., the plants are placed in water.

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| <i>Elodea canadensis.</i>                                                             | After 3-4 hours in 35 p. c. or 6-8 hours in 30 p. c. of cane-sugar shows, when examined in 20 p. c., no power of assimilation, though most cells remain living. In 10 p. c. a weak evolution of O is shown in most cases, which after 1-2 hours in water becomes normal.                                                                                                                                                                       |
| <i>Catharinea undulata</i> ;<br><i>Dicranum scoparium.</i>                            | After 12 hours in 35 p. c. or 24 hours in 30 p. c. no evolution of oxygen is shown under optimal illumination. Recovery as in <i>Elodea</i> .                                                                                                                                                                                                                                                                                                  |
| <i>Bryum caespitium</i> ;<br><i>Grimmia conferta</i> ;<br><i>Orthotrichum affine.</i> | After 1 day in 35 p. c., show in 20 p. c. no evolution of O, but assimilation returns on accustoming to water, and becomes normal in a few hours. After 1 day in 30 p. c., show in 20 p. c. weak evolution of O in many cases, but in most cases none. After 2 days in 30 p. c. some leaf-cells die; rest show no evolution of O. On accustoming to water a weak evolution of O is shown, which in 1 day is fairly active in all living cells. |

In all cases it is impossible to produce a complete temporary stoppage of the power of assimilation without killing a few leaves or leaf-cells, though most remain living and recover. The different times necessary for the stoppage of assimilation to take place are probably due to differences in the permeability of the cell-wall and in the force with which the cell retains water. Thus *Elodea* is very readily plasmolysed, but *Catharinea* and *Dicranum* much less readily, and *Bryum* and *Grimmia* least readily. When the concentration of the cell-sap reaches a percentage equivalent to 35 per cent. of sugar, assimilation soon



ceases (external action); but, according to Saposchnikoff, in Phanerogams, when sugar accumulates in the cell-sap as a product of assimilatory activity, as soon as the latter contains 8 per cent. of glucose further assimilation ceases (internal action).

The stoppage of assimilation which occurs when the maximal accumulation of carbohydrates is reached, is undoubtedly largely due to the fact that the sugar can no longer be converted into starch and accumulates in the cell-sap till the assimilatory limit is reached, but is also partly a direct effect of the accumulation of starch in the chlorophyll grain, for, as will be seen later, in certain cases, under circumstances in which no large amount of sugar can be present, an excessive deposit of starch in the chlorophyll grain is accompanied by a cessation of the power of assimilation. Dehnecke came to the conclusion, from the well-known fact that in many chlorophyll grains a large deposit of starch may take place, though the amount of assimilation they can possibly have carried on can only have been trifling, that green chlorophyll grains, which have as their sole function that of a leucoplastid and have no power of assimilating, are of common occurrence in green plants.

*Pellionia Daveauana* affords very good material for testing the truth of Dehnecke's conclusions. In the stem of this plant the parenchyma-cells contain special small amyliiferous chlorophyll grains. In the young stems and petioles the chlorophyll grains in the outer half of the stem contain little or no starch; the central cells are larger, and in them each chlorophyll grain forms a green plate or flattened cap on the end of a large starch grain. The chlorophyll grains are, when without starch, round and about  $\frac{1}{3}$  to  $\frac{1}{2}$  the size of those of the leaf mesophyll, and each cell of the stem parenchyma contains from six to a dozen. The outer cells of the stem contain about  $\frac{1}{15}$  to  $\frac{1}{20}$  the amount of chlorophyll that the leaf-cells do. Nevertheless, from them on exposure to light a weak evolution of oxygen can be detected, but none from the inner starch-loaded cells. In the older basal parts of the stem all the parenchyma is amyliiferous, the starch grains being much larger and almost entirely filling up the cavity of the cell. Here no power of assimilation can be detected.

The question is, whether with the removal of the starch the power of assimilation returns to these leucoplastic chlorophyll grains. To remove the starch, the plants may either be kept in the darkness or exposed to light in an atmosphere free of  $\text{CO}_2$ . In the latter case, after 10 days' exposure at  $20^\circ \text{C.}$  to  $25^\circ \text{C.}$



starch is still present in the cells immediately around the vascular bundles, but the grains are more or less corroded and lessened in number. Here no evolution of oxygen is shown, but in some of the outer parts of the stem a very weak power of assimilation can be detected. After longer exposure all the green parts, including the leaves, lose the power of assimilation, and the plants finally die without any further marked decrease in the amount of starch present taking place.

The most effective mode of removing the starch, which shall at the same time cause the least possible injurious influence, is to keep the plant at a fairly high temperature exposed to extremely weak diffuse daylight in an abundant and frequently-renewed supply of moist air. After being under such conditions for 3 weeks, or in darkness for 2 weeks at 30° C., the plants are quite healthy, starch has entirely disappeared, the chlorophyll grains of the stem are quite normal, round, green, and show, if examined after a few hours' exposure to light, a moderately active evolution of oxygen, which is normal for the amount of chlorophyll present.

If the stems are examined after a day or two in darkness, the starch grains show as yet no signs of dissolution, and the amount of sugar present in the cells must be very small, but no power of assimilation is shown. The stoppage of assimilation in these leucoplastic chlorophyll grains is due simply to the accumulation of starch; they retain a potential power of assimilation which is capable of being exercised as soon as the inhibitory influence exercised by the deposited starch is removed.

In all the cases in which Dehnecke concluded that non-assimilatory chlorophyll grains having solely the function of amyloplasts were present (pith of *Phaseolus* and *Pisum*, chlorophyllaceous cotyledons, &c.), it is only when the chlorophyll grain is overloaded with starch that any stoppage of the power of assimilation takes place. He cites Hanstein's observations upon the amyliiferous chlorophyll grains of the medullary cells of *Chara*, and Sachs upon the chlorophyll "Bläschen" in the flesh of fruits as proving that the chlorophyll grains in these cases also are non-assimilatory. The medullary cells of *Chara* have, however, been shown (pp. 417, 439) to possess a power of active assimilation. If tangential sections from the young green fruits of *Pyrus baccata* and *P. Torringo*—through the outside layers in which the chlorophyll grains are abundant, green, and normal—are examined, they are found to show a distinct though



not strong evolution of oxygen when exposed to light. As soon as the chlorophyll grains are loaded with starch and when converted into "Bläschen" or disintegrated they naturally cease to assimilate. Dehnecke erred in supposing that amyliiferous leucoplastic chlorophyll grains never could assimilate; the stoppage of assimilation is more accidental than functional, and in many cases of merely temporary duration.

In many young stems the chlorophyll grains are found in the form of thin green envelopes enclosing one or more starch grains. These are, according to Dehnecke, non-assimilatory chlorophyll grains from which the starch grains finally escape, leaving the thin green envelope as a chlorophyll "Bläschen," which finally disappears, new secondary chlorophyll grains being formed by protoplasmic differentiation. In investigations of this kind the effects of manipulation and section-cutting are liable to form a serious source of error, for the latter especially may cause sections of living tissue to show chlorophyll grains as "Bläschen" which were originally in the intact tissue perfectly normal (see p. 390). When, after the starch disappears, new normal chlorophyll grains are seen, these are not new formations produced by protoplasmic differentiation, but are formed from the original starch-bearing chlorophyll grains. Thus in the living uninjured cells it can commonly be seen that one side of the "Bläschen" is thicker and distinctly green. In other cases this thickening forms a distinct but irregularly-shaped chlorophyll grain, which can finally be seen without any starch-enclosing envelope, but with the impression of where a starch grain has previously been on its inner concave surface. In *Pellionia* the amyliiferous chlorophyll grains of the stem are reduced to thin, flattened, green plates, which are often almost invisible, but when the starch is removed they become round, normal, green, assimilating chloroplastids. The "primary" and "secondary" chlorophyll grains are therefore simply two different conditions of the same chloroplastid. In fruits, after the starch grains have been converted into sugar the chlorophyll "Bläschen" finally disintegrate, but in all cases where normal chlorophyll grains appear after the disappearance of the starch they are formed from chloroplastids previously present. The initial power of forming chloroplastids by protoplasmic differentiation seems to be present only in the embryonic condition of the cell and to be lost in post-embryonic and adult life.



*Effects of Insolation upon Assimilation.*

It is well known that prolonged exposure to direct sunlight is fatal to Bacteria and many Algæ, and exercises a marked injurious influence upon many shade-loving and water-plants. Even the most resistant of Phanerogams are, especially at certain periods of development, unable to stand prolonged and severe exposure with impunity. Before the vitality of the plant is permanently affected, there appears to be an intermediate condition from which perfect recovery is possible, but during which the power of assimilation is in abeyance though the chlorophyll apparatus is green and intact. Experiments in this direction were performed on both water- and land-plants, the chief obstacle being the difficulty of obtaining sufficiently long periods of exposure to produce the desired effect.

Plants of *Elodea*, *Utricularia*, and *Chara* exposed to full sunlight in water kept cool by constant renewal from 5 A.M. to 5 P.M., during a bright unclouded day, gave the following results:—

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| <i>Elodea canadensis.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                         | Nearly all leaves and leaf-cells living and green; in most cases weak assimilation, but in many leaves, though all cells are living, none. After 2 hours in diffuse daylight, all living leaves show a more or less active assimilation.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <i>Utricularia vulgaris.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                      | All plants living and showing weak assimilation, which is, however, absent from many branches. After 2 hours in diffuse light, fairly active assimilation in all cases.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <i>Chara fragilis.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                            | Many cortical and end cells killed and bleached, plenty living, but no assimilation, and rotation absent or slow. In many branches the cortical cells are killed, but the medullary cells remain living and show in 15–30 minutes rapid rotation. If the cortical cells are quite bleached and the medullary chlorophyll grains quite colourless, then the medullary cells die and rotation ceases in a few hours without any return of assimilation taking place; but if the medullary chlorophyll grains retain partly or entirely a greenish coloration, the cell may recover and show, after 3–4 hours in diffuse light, or, where but few grains retain a greenish coloration, only after several days, a distinct evolution of O. In the living end cells rotation is active in 15–30 minutes, and in 1 hour weak assimilation, fairly active in 3. In a few end cells the chlorophyll is more or less bleached, and though rotation may return and continue for from a few hours to several days no return of assimilation takes place, the cells finally dying. |
| Ringed cell preparations of end cells of <i>Chara</i> kept floating on water at about 15° C., exposed to full sunlight for 4 hours, showed neither rotation nor assimilation. After $\frac{1}{4}$ hour in diffuse daylight a faint evolution of oxygen is shown by most end cells, but in some cases still no rotation. In 1 hour the evolution of oxygen is moderately active, but rotation is still slow. In 5 hours both assimilation and rotation are active. |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |



Similar results as regards assimilation were obtained with freshwater Algæ exposed in water kept below 15° C. for 10 hours, the sun being slightly clouded during the first five, but unclouded during the last five hours.

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| <i>Spirogyra</i> ;<br><i>Edogonium</i> . | Many filaments dead, but plenty living and with unbleached chlorophyll, but no assimilation; none after 2 h. in diffuse light, weak in 5 h., and about normal in all living filaments next day.                         |
| <i>Mesocarpus</i> .                      | All cells killed and bleached. After 4 h. exposure to sunlight plenty of living and normal cells show no assimilation, and mostly none after 2 h. in diffuse light. Next day assimilation is shown by all living cells. |

Similarly with shade-loving land-plants after 12 hours' exposure to full sunlight:—

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| <i>Dicranum scoparium</i> . | Leaves living and green, but assimilation absent entirely or from apical part only, and where present weak. After 2-4 h. in diffuse light, assimilation is active.                                                                                                                                                                                       |
| <i>Oxalis Acetosella</i> .  | Leaflets green and living, but drooping, and in some cases slightly wilted. No assimilation shown. After 1-2 h. in diffuse light faint, and in 2-3 h. fairly active assimilation, and the leaflets have re-expanded. Leaflets shaded in the drooping position may show a weak assimilation, but none if a reflecting mirror has been placed behind them. |

The drooping position assumed by the leaflets of *Oxalis* is simply protective; there is no correlation between the assumption of the drooping position and the temporary loss of the power of assimilation. Preparations made at night from the leaves of *Oxalis* when in the drooping nyctitropic position show a normally active power of assimilation, and the same is the case with leaflets of *Mimosa*. The movements performed in assuming the nocturnal nyctitropic position of certain "sleep" plants are not accompanied by any corresponding internal changes or alterations in the power of assimilation. In this respect the sleep of plants is more external and apparent than internal and real.

With sufficiently prolonged exposure stoppages of the power of assimilation can be caused in the leaves of Phanerogamic trees and shrubs.



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| <i>Pyrus Tor-<br/>ringo</i> ;<br><i>Æsculus<br/>flava</i> ;<br><i>Ficus<br/>elastica</i> . | Leaves exposed, attached to the parent plant, to full sunlight till 4 P.M. show no assimilation though living, green, and with normal chlorophyll grains. After 2 h. in diffuse light still no assimilation, but next day the same preparations show, if living, a weak but distinct power of assimilation, and fresh preparations made from the same leaves now show a fairly active power of assimilation. |
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That the stoppage of assimilation is a direct effect of the exposure to sunlight, and is not due to any accumulation of carbohydrates, is shown by the following facts:—(1) On a leaf exposed to bright diffuse daylight no perceptible inhibitory influence is produced, though the actual amount of assimilation must be as great as, if not greater than, in a leaf exposed to full sunlight. (2) The recovery and return of assimilation after insolation will take place in diffuse daylight, and this although the leaf has been separated from the parent plant. (3) After recovery assimilation may remain fairly active for several days in separated leaves kept exposed to diffuse daylight, whereas if the stoppage were due to an excessive accumulation of carbohydrates it should only be weak and should soon cease again; *i. e.* as soon as the amount of sugar &c. removed by respiration during the night is replaced by the renewed assimilation. Nor is the stoppage the result of the heating effect of the sun's rays, for in *Pyrus* and *Æsculus* transpiration suffices to keep the leaf cool during the exposure, and though the thick leaves of *Ficus* may be slightly warmed by the sun's rays, the rise in temperature is only slight and does not approach anywhere near the critical point.

*Ulmus scabra* var. *pyramidalis*, *Acer tataricum*, and *Fagus sylvatica* var. *atropurpurea* seem to be more resistant than the plants previously mentioned. Attached leaves exposed during a cloudless day to full sunlight till 5 P.M. show a quite distinct but weakened power of assimilation, which is after a few hours' exposure to diffuse light, or, in some cases, not till the next day, quite active. The retention of the power of assimilation in such leaves may be due to the fact that for a considerable portion of the day they have their edges to the sun or are exposed only to oblique illumination. Hence it is seldom that under normal conditions, except in shade-loving plants, that even an entire day's exposure to full sunlight causes anything more than a weakening of assimilation. In young leaves, in which assimi-



lation is not yet fully active and the tissues and cuticle thin and transparent, and in leaves several months old which have passed the zenith of their vitality, a temporary stoppage of assimilation is much more readily produced by a full day's insolation, and the leaves may become paler or even yellowish. In normal adult leaves it is best produced by removing all but one or two leaves from a terminal leafy branch, and keeping the surfaces of these perpendicular to the sun's rays for an entire unclouded day. The leaves remain fresh and green, but assimilation is either weak or absent. After 2-3 hours in weak diffuse light, a faint power of assimilation may be shown in the latter, but generally still none. Next day the separated leaves, kept under a bell-jar, with the petioles in water, show active powers of assimilation.

*Quercus pedunculata*, *Tilia europæa*, and *Prunus avium*, of temperate plants, and *Griselinia littoralis*, *Bambusa gracilis*, and *Euonymus fimbriata*, of warm temperate and tropical plants, gave similar results. In exposed cut leaves, with their petioles in water, the stoppage is more readily produced; but here the conditions are abnormal, and the leaf may be more or less wilted after a full day's insolation. Also, even when the leaf remains fresh and green, assimilation may be permanently affected, only a weak evolution of oxygen being shown on recovery. In exposed adult leaves, taken from a variety of plants after a full day's sunlight, assimilation was always present though often weak. In nature leaves are exposed to the full force of the sun's perpendicular rays only for a short time daily. A very slight protection is in most cases sufficient to prevent the stoppage of assimilation, such as a slight clouding over of the sun, continuously or at intervals, or covering the leaf with a film of cigarette-paper, or allowing the edge of the leaf to be turned to the sun. Hence in nature a stoppage of assimilation as a direct effect of sunlight is of very rare occurrence in actively assimilating leaves, though a weakening must often be produced.

The stoppage of assimilation is not necessarily associated with any visible change in either the chlorophyll or the plasma. When green leaves are exposed to sunlight the decomposition of chlorophyll goes on more rapidly than its production, though the amount of chlorophyll decomposed is insufficient to cause a change in coloration visible to the naked eye. Keeble\* has

\* Keeble, "Hanging Foliage of certain Tropical Trees," in *Annals of Botany*, vol. ix. 1895, pp. 59-93.



shown that young leaves of *Amherstia nobilis* exposed horizontally to full sunlight formed on extraction with alcohol a weaker solution of chlorophyll than shaded leaves or leaves kept in the normal drooping position did.

Pringsheim \* has shown that concentrated sunlight exercises an oxydative photo-chemical effect upon plant-cells and chlorophyll, bleaching the latter, and inducing in the cell-plasma a condition of light rigor, finally followed by death.

Thus exposure to concentrated sunlight, precautions being taken to avoid all heating effect, and hence to obtain a purely photo-chemical action, causes the rotation of protoplasm to be slowed, and finally stopped in a condition of light rigor, from which at first recovery is possible, but not later (after 5 to 10 minutes' exposure). It is possible to produce a condition of permanent light rigor, *i. e.* death, in the chlorophyll grains over an exposed region of a cell of *Chara* without affecting the vitality of the cell, *i. e.* the plasma of the chlorophyll grain appears to be more sensitive than the general protoplasm of the cell. Hence the temporary cessation in the power of assimilation of chlorophyllaceous cells, which may be caused by prolonged and continuous exposure to direct sunlight, is probably due to a photo-chemical effect upon the plasma of the chlorophyll grain, producing in it the initial stages of light rigor, associated with a temporary inability to assimilate, the action being similar to the paratonic influence of light upon growth, and the condition induced in the chlorophyllous plasma being perhaps analogous with the condition of immotility which may be produced in the protoplasm of irritable organs by the action of various chemical and physical agencies.

However the hindrance or stoppage of assimilation may be produced, the fact that such may occur as the result of prolonged exposure to direct sunlight gives additional interest to the different means of protection which various plants possess against the action of light. In temperate regions it is only on very rare occasions and under exceptional conditions of exposure that any marked influence upon assimilation is produced in healthy adult leaves, and hence here protective agencies are almost entirely absent. The leaflets of the shade-loving *Oxalis Acetosella*, however, when exposed to sunlight assume a drooping

\* Pringsheim, "Zur Theorie der Assimilation, Chlorophyllfunction und Lichtwirkung," in Pringsh. Jahrb. Bd. xii. 1882, pp. 326-344.



position. The more delicate young leaves are very commonly protected either by being shaded by older leaves, or by a hanging position, or by the formation of a red dye. In tropical plants, where the exposure is much more intense, the abundance and importance of the protective agencies is correspondingly increased. In many tropical plants the leaves place themselves obliquely to the sun's most intense perpendicular rays; but most common of all is the formation of a red dye in either the epidermal cells or in the mesophyll cells as well. Johow\* has shown that the formation of the red dye is directly due to the action of light, the exposure acting as a stimulus on certain leaves, and causing in them a formation of red dye. In very many cases this irritability disappears as the leaf becomes adult, and the formation of the dye having ceased, that which is already present in the cell is soon decomposed or absorbed. Engelmann† has shown that the red dye allows nearly all the rays absorbed by chlorophyll to pass through, cutting off for the most part only the rays which are of little or no use for assimilation. The greatest absorption takes place in the yellow and yellow-green, and a lesser but still large amount in the blue, the resultant light being red. The amount absorbed by the red sap may amount to  $\frac{1}{3}$  or  $\frac{1}{2}$  of the incident light, and it is just those rays which, according to Pringsheim, produce the greatest photochemical effect which are thus removed.

Keeble supposes that one use of the red dye may be as a protective influence against the heating effect of intense sunlight. There can be no doubt, however, that transpiration is by far the most important agent in keeping the leaf at a low temperature; and, as Keeble himself shows, the difference of temperature produced by the presence of the dye is very slight, and does not amount to more than from 1° C. to 2° C. Pick‡ concludes that certain rays of light hinder the translocation of starch, and that the red dye is of importance in shielding the leaf from such rays. In support of this contention he argues

\* Johow, "Ueber die Beziehungen einiger Eigenschaften der Laubblätter zu den Standortsverhältnissen," in Pringsh. Jahrb. Bd. xv. p. 299.

† Engelmann, "Die Farben bunter Laubblätter und ihre Bedeutung für die Zerlegung der Kohlensäure im Lichte," in Bot. Zeit. 1887, Nos. 27 & 28.

‡ Pick, "Ueber die Bedeutung des rothen Farbstoffes bei den Phanerogamen und die Beziehungen desselben zur Stärkewanderung," in Bot. Centralblatt, xvi. pp. 9-12.



that (1) as the red colour disappears and the chlorophyll grains become green, starch becomes more abundant in the chlorophyll grain, because light now exercises its full inhibitory action upon the translocation of starch; (2) young red leaves of *Quercus pedunculata* &c. contain plenty of starch before the chlorophyll grains become green, the starch being derived by excessive translocation from the parent plant, owing to the rays inhibitory to translocation being cut off; and (3) in red light starch is formed in the mesophyll but not in the palisade parenchyma, owing to the red light allowing more rapid translocation from the palisade parenchyma cells. The abundant formation of starch in the chlorophyll grain as it becomes fully adult is simply due to the increased activity of assimilation (see pp. 453-4), and is not in any way related to the disappearance of the red dye, for the same phenomenon is shown by leaves which remain red when adult, provided assimilation is sufficiently active. Similarly with (2), the deposition of starch in young-developing chlorophyll grains is in no way related to the presence of a red-colouring material in the cell-sap, and takes place equally well in young leaves in which all red colour is absent. In (3) the phenomenon is due to the illumination, and hence also the assimilation, being weaker than normal. In such cases sugar does not accumulate in the palisade parenchyma in sufficient amount to reach the starch-forming percentage; but in the mesophyll, especially around the veins, owing to the narrowing of the channel through which it passes, the sugar accumulates, reaches the starch-forming percentage, and starch grains appear in the mesophyll cells.

None of the observations cited by Pick form a certain proof that the special importance of the red dye is due to its cutting off those rays of light which hinder or prevent the translocation of carbohydrates. The function of the red dye seems to be mainly to protect the plasma against the photo-chemical action of strong sunlight. When the red dye is present, as is often the case, over the veins and conducting tissues only, it will, as Johow has shown, by virtue of this protective influence aid translocation in general. Over the assimilatory portion of the leaf, though it appears to exercise a slight protective influence against the heating effect of the sun's rays, and also helps to protect the chlorophyll against the decomposition induced by the action of sunlight, the main function of the red dye is to protect



the assimilatory cells against those rays of light which under intense and prolonged exposure tend to induce in the plasma, more especially of the chlorophyll grain, a condition of light rigor, and thereby diminish or inhibit their power of assimilation.

*Assimilation in Parasitic Plants with Normal or Modified Chlorophyll.*

Bonnier\* has recently worked on the assimilation of green-root parasites as exemplified among the Scrophulariaceæ, and finds that *Melampyrum* can assimilate  $\frac{2}{3}$ , *Pedicularis* and *Rhinanthus*  $\frac{1}{5}$ , and yellow-green individuals of *Rhinanthus* and *Bartsia* only  $\frac{1}{12}$  the amount of CO<sub>2</sub> that the non-parasitic *Veronica* can. In the last case it is only under strong illumination that assimilation is greater than respiration, whilst in *Euphrasia* and in some plants of *Rhinanthus* and *Bartsia*, even by strong illumination, no evolution of oxygen can be detected. The mere fact, however, that the power of assimilation is much less active in one plant than in another from an allied genus is by no means a sufficient proof that the former plant obtains a portion of its carbohydrates parasitically, but may be simply due to the fact that the amount of carbohydrate material consumed or required is less in the former case than in the latter. The yellow-green individuals of *Rhinanthus* and *Bartsia*, which have almost no power of assimilation, and in which almost complete parasitism is supposed to exist, are in an abnormal condition, and are not fit subjects for experimentation. As regards *Euphrasia*, Bonnier is certainly in error. The chlorophyll grains of this plant are large, green, and normal, and all living chlorophyllaceous cells, not only in *Euphrasia officinalis* but also in *Rhinanthus Crista-galli* and *Bartsia Odontites*, show, on examination by the Bacterium method, an active power of assimilation.

Bonnier's error may arise from the fact that the cut plants kept with their stems in water are very liable to be injuriously affected. Thus after 6 hours' exposure to full sunlight quite unwilted plants show no perceptible or only a very weak power of assimilation, which is, however, after a night in darkness, again present and fairly active in all living leaves. If the plants

\* Bonnier, "Sur l'assimilation des plantes parasites à chlorophylle," in *Comptes-rendus*, Paris, 1891, t. cxiii. p. 1074.



are left *in situ* on the turf from which they are growing, all parts above ground except the *Euphrasia*-stems being removed, and the turf kept moist during the exposure, the conditions are more normal and the plants more resistant; but after a day's exposure to full sunlight, the fully exposed leaves, though green, living, and apparently normal, show none or only a weak power of assimilation.

All cells, whether from parasitic or non-parasitic plants, which contain normal chlorophyll grains possess the power of assimilation, and the intensity of the assimilation bears a direct ratio to the amount of chlorophyll present. The amount of assimilation is of course dependent upon the intensity and duration of the illumination, but is also owing to the fact that the rate at which the assimilatory products are removed exercises an important influence upon the power of assimilation, ultimately dependent upon the special internal structure and vital peculiarities of each individual plant, which are in no two cases precisely similar.

Even in plants in which neither the colour nor the form of the chlorophyll grains is normal, a marked power of assimilation may still be present. Thus Temme\* has extracted chlorophyll from *Cuscuta europæa*, though it is only present in relatively small amount. By passing an assimilatory mixture of H and CO<sub>2</sub> over the plants when exposed to sunlight, and using the oxidation of phosphorus as a test for the presence of oxygen, he succeeded in detecting an evolution of oxygen from them. The experiments are, however, not conclusive as to whether the oxygen evolved is produced on exposure to light or was previously present in the tissues of the plant. The green-colouring material, according to Temme, exists partly in the uniformly green plasma, or in separate plasma masses or in grains, this last form being only found in the flower-heads. Detailed and conclusive observations can be made only by means of the Bacterium method, the following being the results thus obtained with *Cuscuta Cephalanti*:—

\* Temme, "Ueber das Chlorophyll und die Assimilation der *Cuscuta europæa*," in Ber d. D. Bot. Gesell. 1883, Bd. i. p. 485.



*Cuscuta Cephalanti.*

1. Young pale-yellow seedlings, a few cm. long. Chlorophyll grains faint greenish yellow. No evolution of oxygen shown in light.
2. Young plants, with well-formed thick coils, with plenty of haustoria. Colour is brownish yellow. Epidermal cells, starch almost entirely absent, chlorophyll grains small and deeply reddish brown, weak evolution of oxygen. Cortex, brownish-yellow chlorophyll grains, starch abundant, frequently 2 or 3 grains to one chlorophyll body; moderately active evolution of oxygen. Inner cortex, chlorophyll grains yellowish green, starch as before; evolution of oxygen slightly more active than from outer cortex.
3. Plants over 3 months old. Chlorophyll grains brownish red. For most part each has formed a group of starch grains, between which the body of the chlorophyll grain is seen only as a reddish-brown matrix. In cells quite packed with starch, generally no power of assimilation can be detected; but where less starch is present (outer parts of older and all parts of younger stems), a weak to moderately active evolution of oxygen is shown. Young buds 0.3 cm. long, from the axils of the scales, contain distinct round small pale yellowish-green chlorophyll bodies; but no evolution of oxygen is shown. When 1 cm. long or more, the chlorophyll grains are yellowish to greenish brown, and show distinct but not strong assimilation.

*C. Cephalanti* therefore possesses a distinct power of assimilation, which commences as soon as the chlorophyll grains are mature, and continues after all green coloration has been masked by the formation of a reddish-brown pigment, which is probably a derivative of chlorophyll\*. Under favourable conditions the bulk of the *Cuscuta* plants was much greater than that of the plants (*Convolvulus*) on which they were parasitic.

\* In such cells the nucleus may often be seen to be coloured faintly brownish whilst the cell is still living, and in dead cells the coloration generally deepens. Campbell ("The Staining of Living Nuclei," Arbeit. Bot. Inst. Tübingen, Bd. ii. 1887) has shown that the living nucleus may be stained by certain aniline dyes. The above case is interesting as being an example of a normally stained living nucleus. The shape of the nucleus also is often peculiar; it may be acicular, spindle-shaped, or stalked, with a club-shaped extremity projecting into the lumina of the cell, and the stalk may be longer than the diameter of the cell, in which case it is coiled in one or two turns. The nuclei of *C. europæa* rarely show the same peculiarities of form, but are generally round or oval and normal in shape.



*Cuscuta europæa.*

- (a) Young plants 30 to 40 cm. long. Apex is greenish yellow; but chlorophyll grains almost colourless, the apparent deepness of the coloration being due to the transparency of the young tissues. Starch grains few or none. No assimilation. 1st internode: pale greenish yellow; chlorophyll grains faint yellow, more starch; very weak evolution of oxygen. 2nd internode: paler in colour, but chlorophyll grains more deeply greenish yellow; starch abundant; moderately active evolution of oxygen. 3rd internode: red, due to dye in sap of epidermal cells; rest as in 2nd internode. 4th internode: coiled several times round nettle-stem and with plenty of haustoria; starch very abundant, but otherwise as in 2nd internode. The colour of the chlorophyll grains is not very deep, but they are distinct and separate when no, or only one, starch grain is present. When several starch grains are formed, the outline of the chlorophyll grain is generally lost, the group of starch grains appearing simply to be united by a yellowish matrix in which they are imbedded.
- (b) Stems from adult flowering plants. In outer cortex, less starch, fewer chlorophyll grains, and weak evolution of oxygen. In inner cortex, starch and chlorophyll grains more abundant, and fairly active evolution of oxygen. From cells quite packed with starch, no evolution of oxygen is perceptible. The chlorophyll grains may fuse to form an irregular coloured amyliiferous mass, and are rarely spindle-shaped instead of round or oval.
- (c) Inflorescences. In the outer tissue forming the walls of the anthers normal small green chlorophyll grains are present, and a weak to moderately active assimilation is shown until after dehiscence. In the large cells of the adherent calyx the chlorophyll grains are few in number, but moderately large and distinctly green, and a weak but distinct evolution of oxygen is shown. The central carpellary portion is much greener. The chlorophyll grains have a tendency to fuse to form large green irregular masses with or without starch grains. More than one such mass may be present in the cell, but the rest of the plasma is colourless. Plenty of cells contain quite normal, but somewhat pale green chlorophyll grains. Fairly active evolution of oxygen, whether the grains are single or fused, and with or without starch. From some cells quite packed with starch, the evolution of oxygen is weak or imperceptible.

The power of assimilation exhibited by the chlorophyllaceous stems of *C. europæa* is weaker than in similar green stems of *Convolvulus*, but not more so than would be expected from the less amount and weak intensity of coloration of the chlorophyll bodies. In the chlorophyllaceous parts of the flower the power of assimilation is quite as active, if not more active, than is generally the case in such parts. Wiesner\* has shown that in *Neottia Nias-avis* and in the *Orobanchæ* distinct chlorophyll

\* J. Wiesner, "Untersuchungen über die Farbstoff einiger für chlorophyllfrei gehaltenen Phanerogamen," in Pringsh. Jahrb. Bd. viii. 1872, p. 575.



grains are present, though not in abundance, the green colour of which is often masked by a red or brown pigment. There can be no doubt that these plants also possess a power of assimilation corresponding to the amount of chlorophyll which they contain.

Klebs\* describes an interesting condition which may be produced in *Elodea* and *Funaria* as the result of cultivation in a plasmolytic solution of sugar. This consists in a degeneration and, finally, complete disorganization of the chlorophyll bodies, the plants now being reduced to the condition of obligate parasites. In *Funaria* the alteration appears to be due to the presence of a trace (0.05 p. c.) of  $K_2CrO_4$  in the solution of sugar employed; but in *Elodea*, kept in darkness in water, it goes on as actively as in a solution of sugar. In the sugar solution, according to Klebs, the change occurs more rapidly in light than in darkness. It consists primarily in a reduction in size of the chlorophyll grains, these becoming at the same time yellowish in colour. The degenerative changes finally convert the chlorophyll grains into small red dots, to which frequently a small starch grain is attached. From this condition no regeneration appears to be possible; but if the chlorophyll grains are yellow or yellowish brown they may, under proper conditions, recover and become green and normal again. Klebs assumes that this degenerative change is accompanied by a loss of the power of assimilation, an assumption which is not justifiable without direct experimental proof.

The readiest mode of causing the alteration in the chlorophyll grains of *Elodea* was found to be by keeping the plants in well aerated water at 30° C. and in darkness, when, after a week, nearly all leaves and leaf-cells remain living; but the chlorophyll grains are found in all stages of degeneration from quite small brown grains to green and almost unmodified chlorophyll bodies. As soon as the chlorophyll grains begin to diminish in size and turn yellowish green, the power of assimilation becomes very weak. When they are reduced to from  $\frac{1}{3}$  to  $\frac{1}{2}$  their normal size and have become brownish green, or brownish yellow, the leaf-cells lose all power of evolving oxygen on exposure to light, and this is naturally also the case when the grains are quite small and brown or brownish red. Many leaves show large patches of

\* G. Klebs, "Beiträge zur Physiologie der Pflanzenzellen," in Bot. Untersuch. Tübingen, Bd. ii. Hft. 3, 1888, p. 557.



living but non-assimilating cells, in which the chlorophyll grains are but little smaller than normal; and though many have a brown or brownish-green coloration, many are merely yellowish green and more green than yellow. On re-exposure to light, chlorophyll grains reduced to  $\frac{1}{3}$  their normal size and quite brown or yellowish brown, became green and of normal size in 10 to 30 days. If the reduction has gone further than this, no recovery or regeneration takes place, and in water such cells soon die, though in a nutrient solution they may remain living for days or even weeks without any further change taking place. As recovery takes place, many cells with chlorophyll grains  $\frac{1}{3}$  to  $\frac{1}{2}$  normal size, and still quite yellowish brown, may show distinct but weak assimilation; most cells with the chlorophyll grains  $\frac{2}{3}$  normal size, and brownish or yellowish green, show a moderately active power of assimilation. The stoppage of the power of assimilation is not merely due to the change in colour of the chlorophyll grains, but is primarily due to some internal invisible alteration, of which the change in the colour of the chlorophyll grain is the outward and visible sign; for on re-exposure to light, a weak power of assimilation may return before any perceptible alteration in either the size or the colour of the chlorophyll grain has taken place.

In plants kept in 20-per-cent. sugar solution the same degenerative changes take place, but only with from  $\frac{1}{3}$  to  $\frac{1}{4}$  the rapidity under the most favourable conditions with which it occurs in darkness in water. In sugar solution the degenerative changes are much more rapidly produced when the plants are exposed to bright light, with an abundant supply of oxygen, than when kept in the darkness and imperfectly aerated. Under the latter conditions, after two months the chlorophyll grains may be still green and apparently normal, but show no power of assimilation. Under the former conditions, cells in which the chlorophyll grains are reduced from  $\frac{1}{3}$  to  $\frac{2}{3}$  their normal size and are brownish green in colour, generally show a distinct though weak power of assimilation; but assimilation is always absent when the chlorophyll grains are quite brown and small. The same phenomena of recovery are shown as previously if the plants are accustomed to weaker solutions, and finally to water.

The degenerative changes induced in the chlorophyll bodies by keeping the plants in darkness in water are undoubtedly starvation phenomena, the material used in respiration being supplied



from the integral substance of the cell itself as soon as all reserve carbohydrate, which is never very abundant in actively vegetating *Elodea* plants, is consumed. The same causes which accelerate respiration (fairly high temperature, abundant supply of oxygen, &c.) also hasten the production of the degenerative changes. In sugar solution the change cannot possibly be a phenomenon of starvation, though it may be due to malnutrition of the cell. Very possibly it may be a pathological condition induced by the abundant supply of sugar, the now useless chlorophyll bodies wasting away and degenerating, especially if subject to the changes and decompositions caused by exposure to the action of light.

*The Age of the Leaf Cell or Chlorophyll Grain at which  
Assimilation commences.*

The power of assimilation of a chlorophyll grain when once fully formed in an adult cell remains active under normal conditions during the life of the cell, and only begins to diminish when the vitality of the cell is on the wane. The division of a chlorophyll grain, as has already been seen, to form two new grains does not involve any cessation or diminution of the power of assimilation. When, however, the chlorophyll bodies are being formed in a developing tissue, *ab initio*, by protoplasmic differentiation, they are at first colourless and necessarily without any power of assimilation. To determine more exactly the age of the leaf or leaf-cell at which the chlorophyll grains become capable of assimilating, a series of observations were carried out in spring upon the young developing leaves of a variety of plants. When the leaf is thin and the intercellular spaces few and small, a portion of the lamina free from veins may be examined; but in all other cases sections are used. Whenever no power of assimilation can be detected, the observations are repeated. The measurements given are in terms of the largest adult leaf; thus L.  $\frac{1}{3}$ , B.  $\frac{1}{4}$ , P.  $\frac{1}{9}$  means that the length and breadth of the lamina and the length of the petiole of the young leaf bear to those of the adult leaf these relations. In the first column the measurements are given of the largest leaf in which no power of evolving oxygen can be detected, the subsequent columns tracing assimilation from its first appearance until it is fully active. Where not otherwise stated, the measurements and determinations at any given time are taken from the



|                                           | Under optimal illumination.<br>No evolution of Oxygen.                                                  | Very weak to rather weak power<br>of Assimilation.                                                      | Moderately to fairly<br>active Assimilation.                                                | Quite active<br>assimilation.                                                     |
|-------------------------------------------|---------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| <i>Lonicera tatarica</i> .....            | April 14. Leaves yellowish green.<br>L. $\frac{1}{2}$ ; B. $\frac{1}{6}$ ; P. $\frac{1}{3}$ .           | April 19. Still yellowish. L. $\frac{1}{8}$ ; B. $\frac{1}{9}$ .                                        | April 19. L. $\frac{1}{8}$ ; B. $\frac{1}{6}$ .                                             | April 19. Quite green leaves.<br>L. $\frac{1}{4}$ ; B. $\frac{1}{3}$ .            |
| <i>Lonicera alpigena</i> .....            | Ap. 14. Yellowish green. L. $\frac{1}{4}$ ;<br>B. $\frac{1}{4}$ ; P. $\frac{3}{8}$ .                    | Ap. 19. Yellowish green. L. $\frac{2}{7}$ ;<br>B. $\frac{1}{4}$ .                                       | Ap. 19. L. $\frac{1}{3}$ ; B. $\frac{1}{3}$ .                                               | Ap. 19. Quite green. L. $\frac{2}{7}$ ;<br>B. $\frac{1}{2}$ ; P. $\frac{3}{8}$ .  |
| <i>Ribes nigrum</i> .....                 | Ap. 14. Yellowish green and partly<br>opened. L. $\frac{1}{6}$ ; B. $\frac{2}{11}$ ; P. $\frac{1}{6}$ . | Ap. 19. Yellowish green, and partly<br>opened. L. $\frac{1}{9}$ ; B. $\frac{1}{7}$ ; P. $\frac{1}{8}$ . | Ap. 19. Green and ex-<br>panded. L. $\frac{1}{9}$ ; B. $\frac{1}{7}$ ; P. $\frac{1}{6}$ .   | Ap. 19. Quite green. L. $\frac{2}{9}$ ;<br>B. $\frac{3}{11}$ ; P. $\frac{1}{4}$ . |
| <i>Ribes multiflorum</i> .....            | Ap. 5. Yellowish green and un-<br>expanded. L. $\frac{1}{9}$ ; B. $\frac{1}{7}$ .                       | Ap. 10. Partially expanded, but<br>yellowish. L. $\frac{1}{7}$ ; B. $\frac{1}{6}$ .                     | Ap. 15. Expanded and<br>green. L. $\frac{1}{6}$ ; B. $\frac{1}{8}$ .                        | Ap. 20. L. $\frac{1}{3}$ ; B. $\frac{2}{7}$ .                                     |
| <i>Spiræa sorbifolia</i> .....            | Ap. 10. Yellowish green. L. $\frac{1}{16}$ ;<br>Pinnules, L. $\frac{1}{9}$ ; B. $\frac{1}{10}$ .        | .....                                                                                                   | Ap. 15. Quite green. L. $\frac{1}{11}$ ;<br>Pinnules, L. $\frac{1}{8}$ ; B. $\frac{1}{7}$ . | Ap. 20. L. $\frac{1}{11}$ . Pinnules,<br>L. $\frac{1}{3}$ ; B. $\frac{1}{4}$ .    |
| <i>Daphne Mezereum</i> .....              | Ap. 15. Expanded and yellowish<br>green. L. $\frac{2}{9}$ ; B. $\frac{1}{3}$ .                          | Ap. 20. From central tuft. L. $\frac{2}{9}$ ;<br>B. $\frac{1}{4}$ .                                     | Ap. 20. Expanded leaves.<br>L. $\frac{1}{3}$ ; B. $\frac{1}{2}$ .                           | Ap. 24. L. $\frac{1}{2}$ ; B. $\frac{3}{8}$ .                                     |
| <i>Rosa rugosa</i> .....                  | Ap. 15. Green and expanded.<br>L. $\frac{1}{8}$ . Pinnules, L. $\frac{1}{11}$ ; B. $\frac{1}{6}$ .      | .....                                                                                                   | Ap. 20. Yellowish. L. $\frac{1}{6}$<br>Pinnules, L. $\frac{1}{4}$ ; B. $\frac{1}{3}$ .      | Ap. 20. L. $\frac{1}{4}$ . Pinnules,<br>L. $\frac{3}{11}$ ; B. $\frac{2}{8}$ .    |
| <i>Pyrus prunifolia</i> .....             | Ap. 15. Yellowish green. L. $\frac{1}{8}$ ;<br>B. $\frac{1}{7}$ ; P. $\frac{1}{7}$ .                    | Ap. 20. Partially unrolled. L. $\frac{1}{4}$ ;<br>B. $\frac{2}{11}$ .                                   | .....                                                                                       | Ap. 20. L. $\frac{1}{4}$ ; B. $\frac{3}{11}$ ; P. $\frac{1}{4}$ .                 |
| <i>Syringa vulgaris</i> .....             | Ap. 20. Slightly yellowish. L. $\frac{1}{8}$ ;<br>B. $\frac{1}{7}$ ; P. $\frac{1}{7}$ .                 | Ap. 24. L. $\frac{1}{4}$ ; B. $\frac{1}{3}$ ; P. $\frac{1}{4}$ .                                        | Ap. 30. L. $\frac{1}{2}$ ; B. $\frac{1}{2}$ ; P. $\frac{1}{2}$ .                            | May 4. L. $\frac{1}{2}$ ; B. $\frac{4}{7}$ ; P. $\frac{1}{2}$ .                   |
| <i>Æsculus Hippocasta-</i><br><i>num.</i> | Ap. 17. Leaves unexpanded. P. $\frac{1}{10}$ .<br>Leaflets, L. $\frac{1}{10}$ ; B. $\frac{1}{8}$ .      | Ap. 17. Partially expanded. L. $\frac{1}{8}$ ;<br>B. $\frac{1}{8}$ ; P. $\frac{1}{8}$ .                 | Ap. 22. Quite expanded.<br>L. $\frac{1}{4}$ ; B. $\frac{1}{6}$ ; P. $\frac{1}{3}$ .         | Ap. 25. L. $\frac{1}{3}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{2}$ .                  |
| <i>Acer pennsylvanicum.</i>               | Ap. 17. Buds partly opened. L. $\frac{1}{20}$ ;<br>B. $\frac{1}{16}$ .                                  | Ap. 22. Leaves partly unrolled.<br>L. $\frac{1}{7}$ ; B. $\frac{1}{10}$ ; P. $\frac{1}{8}$ .            | Ap. 25. Leaves expanded.<br>L. $\frac{1}{4}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{4}$ .        | Ap. 29. L. $\frac{1}{3}$ ; B. $\frac{1}{3}$ ; P. $\frac{1}{2}$ .                  |
| <i>Salix alba</i> .....                   | Ap. 17. Buds just opening. L. $\frac{1}{14}$ ;<br>B. $\frac{1}{9}$ .                                    | Ap. 22. Buds quite opened. L. $\frac{1}{7}$ ;<br>B. $\frac{1}{4}$ .                                     | Ap. 25. L. $\frac{1}{4}$ ; B. $\frac{4}{9}$ .                                               | Ap. 29. L. $\frac{1}{3}$ ; B. $\frac{1}{4}$ .                                     |
| <i>Tilia pubescens</i> .....              | Ap. 17. Spring bud-scales opened.<br>L. $\frac{1}{11}$ .                                                | Ap. 22. Leaves still folded and<br>yellowish, L. $\frac{1}{8}$ ; B. $\frac{1}{8}$ .                     | Ap. 25. Expanded and<br>green. L. $\frac{1}{6}$ ; B. $\frac{1}{6}$ ; P. $\frac{1}{4}$ .     | Ap. 29. L. $\frac{1}{4}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{3}$ .                  |
| <i>Magnolia obovata</i> .....             | Ap. 22. Pale yellow. L. $\frac{1}{10}$ ; B. $\frac{1}{7}$ .                                             | Ap. 26. Yellowish green. L. $\frac{1}{7}$ ;<br>B. $\frac{1}{6}$ .                                       | Ap. 30. L. $\frac{1}{3}$ ; B. $\frac{1}{3}$ ; P. $\frac{1}{2}$ .                            | May 5. L. $\frac{1}{2}$ ; B. $\frac{5}{7}$ ; P. $\frac{1}{4}$ .                   |
| <i>Diervilla Lonicera</i> .....           | Ap. 17. L. $\frac{1}{2}$ ; B. $\frac{1}{6}$ ; P. $\frac{1}{3}$ .                                        | Ap. 22. L. $\frac{1}{4}$ ; B. $\frac{1}{6}$ ; P. $\frac{1}{3}$ .                                        | .....                                                                                       | Ap. 25. L. $\frac{1}{3}$ ; B. $\frac{1}{2}$ ; P. $\frac{1}{3}$ .                  |



|                                                     | Under optimal illumination.<br>No evolution of Oxygen.                                                      | Very weak or rather weak power<br>of Assimilation.                                               | Moderately to fairly<br>active Assimilation.                                                                        | Quite active<br>Assimilation.                                               |
|-----------------------------------------------------|-------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| <i>Larix americana</i> .....                        | April 18. Chlorophyll grains very small but green. L. $\frac{1}{4}$ .                                       | April 22. L. $\frac{1}{4}$ .                                                                     | April 26. L. $\frac{1}{3}$ .                                                                                        | April 29. L. $\frac{1}{2}$ .                                                |
| <i>Salix daphnoides</i> .....                       | Ap. 16. Yellowish leaves from central tuft. L. $\frac{1}{12}$ ; B. $\frac{1}{12}$ .                         | Ap. 20. From apical tuft. L. $\frac{1}{7}$ ; B. $\frac{1}{4}$ .                                  | Ap. 25. Exposed green leaves. From L. $\frac{1}{12}$ , B. $\frac{1}{12}$ , to L. $\frac{1}{3}$ , B. $\frac{1}{3}$ . | Ap. 30. L. $\frac{2}{3}$ ; B. $\frac{2}{3}$ .                               |
| <i>Corylus Avellana</i> .....                       | Ap. 19. Buds open, leaves yellowish and folded. L. $\frac{1}{14}$ ; B. $\frac{1}{19}$ ; P. $\frac{2}{15}$ . | Ap. 22. Leaves partly expanded. L. $\frac{1}{3}$ ; B. $\frac{1}{12}$ ; P. $\frac{2}{15}$ .       | Ap. 26. Fully expanded. L. $\frac{1}{3}$ ; B. $\frac{1}{6}$ ; P. $\frac{1}{3}$ .                                    | Ap. 29. L. $\frac{1}{4}$ ; B. $\frac{1}{3}$ ; P. $\frac{1}{2}$ .            |
| <i>Castanea sativa</i> .....                        | Ap. 18. Buds opened, leaves folded. L. $\frac{1}{17}$ .                                                     | Ap. 22. Leaves partly expanded. L. $\frac{1}{4}$ ; B. $\frac{1}{6}$ ; P. $\frac{1}{2}$ .         | Ap. 26. Quite expanded leaves. L. $\frac{1}{4}$ ; B. $\frac{1}{3}$ .                                                | Ap. 30. L. $\frac{1}{3}$ ; B. $\frac{1}{2}$ ; P. $\frac{1}{2}$ .            |
| <i>Quercus sessiliflora</i><br>v. <i>Louettii</i> . | Ap. 24. Buds opened, leaves folded. L. $\frac{1}{3}$ ; B. $\frac{1}{9}$ .                                   | Ap. 28. Leaves expanded. L. $\frac{1}{4}$ ; B. $\frac{1}{3}$ ; P. $\frac{1}{10}$ .               | May 4. L. $\frac{1}{3}$ ; B. $\frac{2}{3}$ ; P. $\frac{1}{4}$ .                                                     | May 10. L. $\frac{3}{4}$ ; B. $\frac{2}{3}$ ; P. $\frac{1}{4}$ .            |
| <i>Betula lenta</i> .....                           | Ap. 24. Buds opened, leaves folded. L. $\frac{1}{8}$ ; B. $\frac{1}{11}$ .                                  | Ap. 27. Leaves expanded. L. $\frac{1}{2}$ ; B. $\frac{1}{8}$ ; P. $\frac{1}{7}$ .                | .....                                                                                                               | Ap. 30. L. $\frac{1}{3}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{4}$ .            |
| <i>Populus tremula</i> .....                        | Ap. 24. Leaves unexpanded, L. $\frac{1}{3}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{9}$ .                         | Ap. 27. Leaf partly unrolled. L. $\frac{2}{3}$ ; B. $\frac{2}{7}$ .                              | Ap. 30. Expanded leaves. L. $\frac{5}{12}$ ; B. $\frac{2}{3}$ ; P. $\frac{1}{6}$ .                                  | May 6. L. $\frac{2}{3}$ ; B. $\frac{2}{3}$ ; P. $\frac{2}{3}$ .             |
| <i>Rhododendron sinense</i> .                       | Ap. 26. Yellowish, with inrolled margins. L. $\frac{1}{4}$ ; B. $\frac{1}{4}$ .                             | .....                                                                                            | Ap. 30. Partly expanded. L. $\frac{4}{9}$ ; B. $\frac{2}{3}$ .                                                      | May 6. Quite expanded. L. $\frac{2}{3}$ ; B. $\frac{2}{3}$ .                |
| <i>Juglans nigra</i> .....                          | Ap. 26. L. $\frac{1}{17}$ . Leaflets, L. $\frac{1}{3}$ ; B. $\frac{1}{4}$ .                                 | Ap. 30. L. $\frac{1}{12}$ . Leaflets, L. $\frac{1}{6}$ ; B. $\frac{1}{3}$ .                      | May 6. L. $\frac{1}{8}$ . Leaflets, L. $\frac{1}{4}$ ; B. $\frac{1}{3}$ .                                           | May 11. L. $\frac{1}{5}$ . Leaflets, L. $\frac{4}{11}$ ; B. $\frac{5}{9}$ . |
| <i>Liquidambar styraciflua</i> .                    | Ap. 26. Leaves folded. L. $\frac{1}{3}$ .                                                                   | May 3. Partly expanded. L. $\frac{1}{7}$ ; B. $\frac{1}{3}$ ; P. $\frac{2}{7}$ .                 | May 9. Fully expanded. L. $\frac{1}{4}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{2}$ .                                     | May 15. L. $\frac{2}{3}$ ; B. $\frac{2}{3}$ ; P. $\frac{4}{7}$ .            |
| <i>Rhamnus grandifolia</i> .                        | Ap. 26. Leaves partly expanded. L. $\frac{1}{9}$ ; B. $\frac{1}{18}$ ; P. $\frac{1}{11}$ .                  | Ap. 30. Lamina pleated and yellowish. L. $\frac{2}{11}$ ; B. $\frac{2}{11}$ ; P. $\frac{1}{3}$ . | .....                                                                                                               | May 6. L. $\frac{3}{11}$ ; B. $\frac{3}{11}$ ; P. $\frac{1}{3}$ .           |
| <i>Fagus sylvatica</i><br>v. <i>atropurpurea</i> .  | Ap. 26. Fully expanded. L. $\frac{3}{14}$ ; B. $\frac{2}{3}$ ; P. $\frac{3}{10}$ .                          | Ap. 30. L. $\frac{1}{2}$ ; B. $\frac{5}{9}$ ; P. $\frac{4}{9}$ .                                 | .....                                                                                                               | May 6. L. $\frac{2}{3}$ ; B. $\frac{2}{3}$ ; P. $\frac{1}{4}$ .             |
| <i>Pyrus nivalis</i> .....                          | Ap. 27. Buds partly opened. L. $\frac{1}{4}$ .                                                              | May 3. Leaves expanded. L. $\frac{1}{3}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{2}$ .                 | May 3. Fully expanded. L. $\frac{1}{4}$ ; B. $\frac{1}{2}$ ; P. $\frac{1}{2}$ .                                     | May 9. L. $\frac{3}{4}$ ; B. $\frac{7}{10}$ ; P. $\frac{2}{3}$ .            |
| <i>Cornus candidissima</i> ...                      | Ap. 27. Buds just opened. L. $\frac{1}{2}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{3}$ .                          | Ap. 30. L. $\frac{3}{11}$ ; B. $\frac{1}{4}$ ; P. $\frac{1}{3}$ .                                | .....                                                                                                               | May 6. L. $\frac{1}{2}$ ; B. $\frac{1}{2}$ ; P. $\frac{1}{2}$ .             |
| <i>Æsculus flava</i> .....                          | Ap. 20. Buds fully opened. P. $\frac{1}{6}$ . Leaflet, L. $\frac{1}{4}$ ; B. $\frac{1}{3}$ .                | Ap. 25. P. $\frac{1}{2}$ . Leaflet, L. $\frac{1}{3}$ ; B. $\frac{1}{4}$ .                        | .....                                                                                                               | Ap. 29. P. $\frac{1}{2}$ . Leaflet, L. $\frac{1}{2}$ ; B. $\frac{1}{3}$ .   |



largest and most fully-developed leaves. It is the age of the young leaf which is of primary importance with regard to its assimilatory powers, and the size of the developing leaf is the readiest, though not always the most accurate, indication of its age. Thus many actively assimilating early developed adult leaves are of smaller size than developing leaves which have not yet commenced to assimilate may be. If, however, at any given date it is always the largest and most fully developed leaves which are examined, this error is largely obviated.

From the table (p. 453-4) it appears that when the foliage is developing in spring, it is only when the leaves have reached a certain size and condition of development, which varies very much in different plants, that any power of assimilation can be detected. If branches are cut towards the end of winter and brought into a warm room with their lower ends in water, the buds in *Syringa* and *Salix* soon open and expand, though in other cases a long latent period intervenes.

|                             | After 2 weeks.<br>No Assimilation.                                                    | After 3 weeks.<br>Weak Assimilation.                                                                               | After 4 weeks.<br>Fairly active<br>Assimilation.                                                     |
|-----------------------------|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| <i>Syringa vulgaris</i> ... | Buds opened. L. $\frac{1}{8}$ ;<br>B. $\frac{1}{4}$ ; P. $\frac{1}{4}$ .              | Chlorophyll grains<br>quite green and<br>fairly large. L. $\frac{1}{8}$ ;<br>B. $\frac{1}{8}$ ; P. $\frac{1}{4}$ . | L. $\frac{1}{3}$ ; B. $\frac{5}{14}$ ; P. $\frac{1}{3}$<br>to L. $\frac{2}{9}$ ; B. $\frac{3}{14}$ . |
| <i>Salix daphnoides</i> ... | Chlorophyll grains<br>small and yel-<br>lowish. L. $\frac{1}{8}$ ; B. $\frac{1}{4}$ . | Quite green. L. $\frac{1}{4}$ ;<br>B. $\frac{1}{4}$ .                                                              | L. $\frac{4}{9}$ ; B. $\frac{3}{4}$ .                                                                |

Comparison with the results in the previous table shows that under these conditions the power of assimilation takes longer to develop, but becomes active whilst the leaves are still relatively small. This is due to the abnormal conditions, and to the fact that the leaves on a cut branch rarely attain their full normal size.

In the young developing leaves of adult plants, perennials, annuals, mosses, &c., the results obtained are very similar to those already given.



|                                                                          | No evolution of Oxygen.                                                                                                                             | Weak but distinct Assimilation.                                                                                                                  | Moderately to fairly active Assimilation.                                                                                                                          | Assimilation quite active.                                                                                                                                       |
|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Oxalis Acetosella</i> .<br>Leaves developing on last year's rhizomes. | Pale yellowish green. Chlorophyll grains less than $\frac{1}{6}$ normal size. P. $\frac{1}{11}$ . Leaflets, L. $\frac{1}{13}$ ; B. $\frac{1}{10}$ . | As before, but chlorophyll grains $\frac{1}{3}$ to $\frac{1}{4}$ normal size. P. $\frac{1}{7}$ . Leaflets, L. $\frac{1}{8}$ ; B. $\frac{1}{8}$ . | Leaflets still folded and yellowish green. Chlorophyll grains $\frac{1}{2}$ to $\frac{1}{3}$ ; P. $\frac{3}{11}$ . Leaflets, L. $\frac{1}{2}$ ; B. $\frac{1}{2}$ . | Leaflets partly expanded and quite green. Chlorophyll grains $\frac{2}{3}$ to $\frac{1}{1}$ . P. $\frac{3}{7}$ . Leaflets, L. $\frac{1}{3}$ ; B. $\frac{1}{3}$ . |
| <i>Phaseolus vulgaris</i> .                                              | Yellowish green. Folded leaflets, L. $\frac{1}{8}$ ; B. $\frac{1}{4}$ . Partially expanded leaflets, L. $\frac{2}{15}$ ; B. $\frac{1}{10}$ .        | Leaflets expanded and faintly yellowish. Chlorophyll grains small, but abundant and green. L. $\frac{1}{5}$ ; B. $\frac{1}{6}$ .                 | Quite green*. L. $\frac{1}{4}$ ; B. $\frac{1}{5}$ (largest terminal leaflet).                                                                                      | Leaflets. L. $\frac{4}{9}$ ; B. $\frac{3}{7}$ .                                                                                                                  |

\* At this stage in the development of the leaf its green colour is darkest. This is owing to the greater transparency of the young leaf, and not to any real greater intensity of coloration. The adult leaf, owing to the development of air-spaces in the mesophyll, becomes less transparent and of a lighter green tint.

*Marchantia polymorpha*.—Gemmæ, average adult diameter (D.) 1 mm. D.  $\frac{3}{4}$ . Quite active evolution of oxygen. D.  $\frac{3}{10}$ . Chlorophyll grains very small, but gemmæ green; distinct but much weaker assimilation. D.  $\frac{2}{10}$  (100–200 cells). Chlorophyll grains extremely small. Margin almost colourless, centre yellowish green, and here just perceptible trace of evolution of oxygen. D.  $\frac{1}{10}$ . Central part distinctly greenish, but no evolution of oxygen.

*Bryum caespitium*.—Length of leaf  $\frac{3}{4}$  adult size. Chlorophyll grains about  $\frac{1}{2}$  normal size and slightly yellowish, but assimilation quite active. L.  $\frac{1}{2}$ ; B.  $\frac{1}{10}$ . Chlorophyll grains smaller and smaller from apex to base of leaf. Apical  $\frac{1}{3}$  fairly active assimilation, middle  $\frac{1}{3}$  weak, basal  $\frac{1}{3}$  none. L.  $\frac{1}{2}$ . Weak but distinct evolution of oxygen in apical  $\frac{1}{4}$  of leaf only. L.  $\frac{3}{8}$ . No evolution of oxygen. Chlorophyll grains distinct and yellowish green in apical portion of leaf, but in basal part almost colourless.

*Dicranum scoparium*.—L.  $\frac{1}{2}$ . Quite active assimilation. L.  $\frac{1}{3}$ . Fairly active assimilation; basal part, chlorophyll grains smaller and weak assimilation. L.  $\frac{3}{12}$ . Protoplasm reticulate and transparent. Apical half, chlorophyll grains nearly normal size, moderately active assimilation; basal half, chlorophyll grains much smaller, assimilation quite weak. L.  $\frac{1}{6}$ . Apical portion, chlorophyll grains fairly large and green, very faint assimilation; basal half, chlorophyll grains smaller and smaller, at extreme base almost colourless, no evolution of oxygen. L.  $\frac{1}{12}$ . No evolution of oxygen. Chlorophyll grains in apical portion yellowish green, and, though small, larger than in base of leaf, L.  $\frac{3}{12}$ , where a weak assimilation is shown.



*Catharinea undulata*.—L.  $\frac{1}{8}$ . Apical  $\frac{2}{3}$ , chlorophyll grains  $\frac{2}{3}$  to nearly full size, and quite active evolution of oxygen. Basal  $\frac{1}{3}$ , chlorophyll grains about  $\frac{1}{6}$  normal size, and weak evolution of oxygen\*. L.  $\frac{1}{6}$ . Apical portion, very weak evolution of oxygen, and chlorophyll grains  $\frac{1}{2}$  to  $\frac{1}{3}$  normal size. L.  $\frac{3}{10}$ . Chlorophyll grains abundant, green, and from  $\frac{1}{3}$  to  $\frac{1}{4}$  normal size in apical  $\frac{2}{3}$  of leaf, but no evolution of oxygen, though the chlorophyll grains twice as large as, and in relation to the protoplasm in greater amount than, in the base of the leaf, L.  $\frac{1}{2}$ .

A careful study of the above results enforces the conclusion that, although the presence of a certain amount of chlorophyll is necessary before any evolution of oxygen can take place, nevertheless, in determining the development of the power of assimilation and the stage at which an evolution of oxygen is possible, equally important factors, of probably plasmatic origin, also enter into play. The leaves of Phanerogams and Mosses and the gemmæ of *Marchantia* are parasitic upon their parent plants for only a relatively short period at the commencement of their existence; for so soon as they show any evolution of oxygen, and this takes place at a comparatively early stage of their life-history, the power of assimilation is greater than that of respiration, and though at first, when the power of assimilation is still very weak, the substance gained during the day may be counterbalanced or more than counterbalanced by that lost by respiration during the night, very soon the amount gained by assimilation far surpasses that lost by respiration, and the leaf, instead of drawing supplies from the parent plant, sends supplies to it.

With moss-leaves it is especially noticeable that in the apical portion of the young leaf, which is more exposed to light and to contact with air, the chlorophyll grains increase more rapidly in size and the power of assimilation is sooner developed than in the more shaded basal portion of the leaf. The same

\* In full-grown leaves of *Catharinea* the chlorophyll grains at the base of the leaf are much smaller ( $\frac{1}{2}$  to  $\frac{1}{3}$  and from  $\frac{1}{4}$  to  $\frac{1}{5}$  in relation to the size of the cell) than those in the rest of the leaf; the evolution of oxygen is correspondingly weaker, and is in some cases almost imperceptible. The cells at the base of the leaf seem to have, owing to their less exposed position and to their forming the channel of communication between the leaf and the stem, as much a conductory as an assimilatory function, and are especially liable to have their assimilatory powers still further diminished by an over accumulation of carbohydrates.



phenomenon may, however, also be shown by the leaves of certain Phanerogams.

*Betula lenta*.—Exposed but folded leaves. L.  $\frac{1}{8}$ , B.  $\frac{1}{10}$ . Green marginal leaf-teeth contain fairly large and green chlorophyll grains and show a weak evolution of O; whereas the central part of the leaf is red owing to the presence of a red sap in the epidermal cells which has disappeared from the more exposed epidermal cells of the leaf-teeth, shows no evolution of O., and the chlorophyll bodies are yellowish-green and from  $\frac{1}{2}$  to  $\frac{1}{3}$  the size of those in the mesophyll of the leaf-teeth. With leaves, L.  $\frac{1}{2}$ , B.  $\frac{1}{8}$ , in which the lamina is still pleated, the same relative differences in the size of the chlorophyll grains of the two parts are still shown, and whilst the body of the lamina shows only a very weak evolution of O, from the leaf-teeth this is moderately active. With fully expanded leaves, L.  $\frac{1}{2}$ , B.  $\frac{1}{4}$ , in which the red colour is present only in the epidermal cells over the veins, the difference between the chlorophyll grains is no longer perceptible, and all parts of the leaf show a quite active power of assimilation.

*Corylus Avellana*.—Leaves, L.  $\frac{1}{13}$ , B.  $\frac{1}{9}$ , folded, hairy, and yellowish green. No evolution of O. Tip of leaf greener and very weak evolution of O. L.  $\frac{1}{2}$ , B.  $\frac{1}{3}$ ; still folded and yellowish green. Tips of the teeth at the ends of the main veins with larger and greener chlorophyll grains and a moderately active evolution of O. Central part, chlorophyll grains smaller and more yellowish; evolution of O absent or barely perceptible, but in parts (over the veins and near the margin) a weak but distinct evolution of O. L.  $\frac{1}{4}$ , B.  $\frac{1}{4}$ , P.  $\frac{1}{3}$ . Leaf expanded. Teeth green, and show active evolution of O. Rest of leaf still slightly yellowish, and chlorophyll grains small and somewhat pale; moderately active evolution of O, which is in part weak. L.  $\frac{2}{3}$ , B.  $\frac{1}{3}$ , P.  $\frac{2}{3}$ . Chlorophyll grains equally large and green, and quite active assimilation in all parts.

In *Betula lenta* there is a very close correspondence between the commencement of assimilation and the disappearance of the protective red dye. In each part of the leaf, as the chlorophyll grains become adult and commence to assimilate, the red dye fades and disappears from the sap of the epidermal cells. In *Corylus*, assimilation appears sooner over the veins than in the rest of the lamina, because these parts are, in the folded condition of the leaf with the ventral surface outwards, more exposed to light and to contact with air than the depressed parts, covered thickly by hairs, lying between the ribs.

It does not necessarily follow that all power of assimilation is absent because no power of evolving oxygen is shown under optimal illumination, for it is always possible that a slight power of assimilation may be present, but is overpowered and masked by the active respiration of the young leaf. To determine whether or not this formed a serious source of error, comparative determinations were made of the amounts of CO<sub>2</sub> given off in



light and in darkness at different stages of their development by certain of the leaves previously examined. The  $\text{CO}_2$  is absorbed by potash, and the decrease in the volume of the receiver gives the amount of  $\text{CO}_2$  evolved, assuming that  $\frac{\text{CO}_2}{\text{O}_2} = \frac{1}{1}$  is the respiratory quotient.

*Corylus Avellana*.—Temperature  $25^\circ \text{C}$ . 5 grammes in A, B, and C.

- A. [Young apices, stipules removed.] Largest leaf, L.  $\frac{1}{13}$ , evolved of  $\text{CO}_2$  in  $3\frac{1}{2}$  hours in bright light 13.1 c.c., in darkness 13.9 c.c.  
 B. Leaves, L.  $\frac{1}{9}$ , B.  $\frac{1}{13}$ , evolved of  $\text{CO}_2$  in  $3\frac{1}{2}$  hours in bright light 6.1 c.c., in darkness 12.7 c.c.  
 C. Leaves, L.  $\frac{1}{4}$ , B.  $\frac{1}{4}$ , evolved of  $\text{CO}_2$  in  $3\frac{1}{2}$  hours in bright light 0.2 c.c., in darkness 8.1 c.c.

*Phaseolus vulgaris*.

- D. 20 leaves, L.  $\frac{1}{8}$ , leaflets L.  $\frac{2}{13}$ , B.  $\frac{1}{10}$ , and partly expanded, evolved of  $\text{CO}_2$  in 3 hours in bright light 1.9 c.c., in darkness 2 c.c.  
 E. 10 leaves, L.  $\frac{3}{4}$ , leaflets L.  $\frac{1}{2}$ , B.  $\frac{1}{6}$ , fully expanded, evolved of  $\text{CO}_2$  in 3 hours in bright light 0.3 c.c., in darkness 2.5 c.c.

In C and E, although assimilation is stronger than respiration, a small quantity of  $\text{CO}_2$  is given off on exposure to light. This is due to the escape and absorption of traces of respiratory  $\text{CO}_2$ , which when once absorbed are no longer available for assimilation, and the same phenomenon is shown by actively assimilating leaves when exposed to bright light. In A, C, D, and E the results coincide with those obtained by the Bacterium method; but in B respiration is apparently twice as active as assimilation, although by the Bacterium method a moderately active evolution of oxygen, which is in parts weak, is shown. This is because the leaves are at this stage folded and quite hairy, and are hence under less favourable conditions for assimilating and evolving oxygen than when examined in the form of sections under optimal illumination by the Bacterium method. Although, theoretically, gas analytical methods should, since they permit the error due to respiration to be allowed for, which is impossible by the Bacterium method, form a more certain test for the presence or absence of the power of assimilation than the latter, as a matter of practice, the Bacterium method is, largely owing to its microscopical and more specialized application, a much more delicate and certain detective agency.



*Conclusions.*

By the operation of a variety of agencies a condition of assimilatory arrest or inhibition may be induced in living chlorophyllaceous cells and tissues. These are: dry heat, moist heat, cold, desiccation, partial asphyxiation, etherization, treatment with acids, alkalies, and antipyrin, accumulation of the carbohydrate products of assimilation, immersion in very strong plasmolytic solutions, and prolonged insolation.

The inability to assimilate is, if the cell remain living, only temporary, being followed sooner or later by a more or less complete recovery of the power of assimilation.

During the whole time in which the power of assimilation is absent, the cell continues to respire. By the direct effect of each particular agency, the respiratory activity may in some cases be but little affected (etherization, over-accumulation of carbohydrates), in one case is increased (moist heat), but in most is diminished (cold, desiccation, immersion in strong plasmolytic solutions). The question, whether or not an after-effect may also be produced on respiration, is unable sensibly to affect the results obtained as regards assimilation. If the inhibition of assimilation experimentally produced be permanent the cell finally dies and ceases to respire.

In the great majority of cases no visible change in the chlorophyll or the chlorophyll grain is associated with the stoppage of assimilation. In such cases the assimilatory arrest probably originates in the plasmatic stroma of the chlorophyll corpuscle, and may be due to some breakage in the necessary vital connection between the assimilatory pigment and the assimilatory plasma.

Most of the above inhibitory agencies operate by checking or arresting the initial stages or primary processes of assimilation (decomposition of  $\text{CO}_2$  and formation of carbohydrate); but since the accumulation of carbohydrate affects assimilation, any cause interfering with the rapidity of its removal from the assimilatory cells may also finally affect their power of assimilation.

Hence in the plant, as a whole, assimilation may be influenced by causes operating upon the general cell protoplasm and affecting the later stages in the process of assimilation (absorption, elaboration, and transformation of the assimilated carbohydrates), either of the assimilatory cell itself or of the cells which are dependent upon it for their supply of carbohydrate



food-material, or by a closure of the channels through which these carbohydrates are removed.

Cells in which the green colour of the chlorophyll grains is quite masked by the presence of a brown or reddish-brown pigment may show a distinct power of assimilation. If the reduction of the chlorophyll be complete, assimilation ceases.

In certain cases isolated chlorophyll bodies may continue to assimilate for a short time after removal from the cell to which they belong.

A developing leaf in which the chlorophyll grains are being formed "*ab initio*" by protoplasmic differentiation lacks at first the power of assimilation. The commencement of assimilation is determined mainly by the development of the chlorophyll pigment, but is also largely influenced by other indeterminate factors, probably plasmatic in origin.

*Postscript.*—The above work was carried out in the Botanical Laboratory of the University of Leipzig during the years 1894 and 1895, under the direction of Professor Pfeffer, to whom my sincere and cordial thanks are due for his able and kindly assistance as well as for his searching but friendly criticism of the facts thereby brought to light.

Leipzig, April 1896.

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## LEBENS- UND BILDUNGSGANG.

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Von Geburt bin ich Engländer. Mein Vater, E. B. Ewart, B.A. (London University), ist Lehrer der Chemie und Director des chemischen Laboratoriums des "Liverpool Institute" (Gymnasium). Ich wurde in Liverpool am zwölften Februar 1872 geboren, bin also jetzt 24 Jahre alt. In meinem 16<sup>ten</sup> Jahr habe ich das "Senior Oxford Local" Examen mit Auszeichnung (*cum laude*) in Chemie bestanden und mir dadurch den Titel "Associate of Arts" von der Universität Oxford erworben.

In demselben Jahr wurde ich als Student der "London University" immatriculirt, und in meinem 17<sup>ten</sup> Jahr habe ich mein erstes Examen zur Erlangung des Diploms als "Bachelor of Science" der Londoner Universität bestanden, darauf im nächsten Jahr "in honours" das Regierungs-Examen in Physik. In demselben Jahr wurde ich Student der "Liverpool University."

Das letzte Examen für das Diplom des "Bachelor of Science" der Londoner Universität bestand ich in meinem 21<sup>ten</sup> Jahre, mit dem Praedicat "*primus, cum laude*" in Botanik. Um diese Zeit war ich während zweier Semester "Demonstrator of Botany" (Botanischer Assistent und Privat Docent) im "University College, Liverpool" (Victoria Universität). In 1894 habe ich zwei Original-Abhandlungen, "Observations on the Vitality and Germination of Seeds" and "Observations on the Growth and Germination of the Pollen-tube," herausgegeben.

In Mai 1894 erhielt ich zur Ausführung wissenschaftlicher Untersuchungen in Botanik ein Regierungs-Stipendium, habe seitdem im Botanischen Institut der Universität Leipzig unter der Leitung des Herrn Geheimrath Professor Dr. Pfeffer gearbeitet und die in dieser Dissertation niedergelegten Untersuchungen ausgeführt.















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